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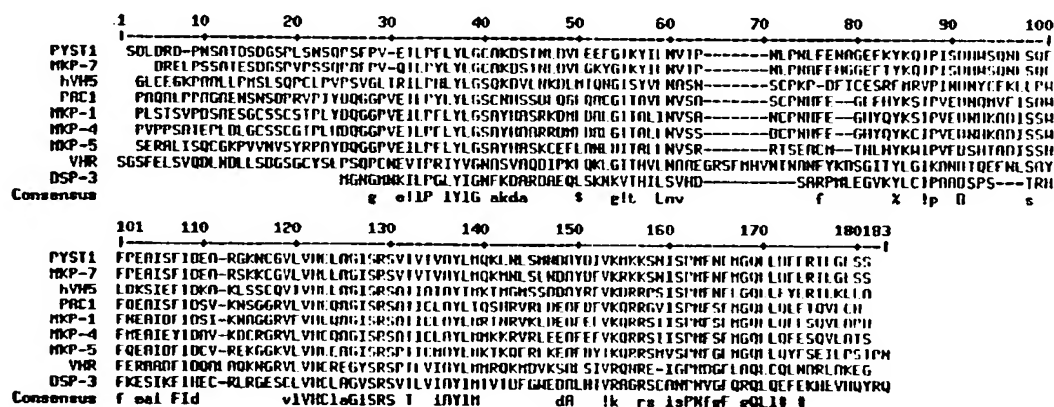
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(54) Title: DSP-3 DUAL-SPECIFICITY PHOSPHATASE



(57) Abstract: Compositions and methods are provided for the treatment of conditions associated with cell proliferation, cell differentiation and cell survival. In particular, the dual-specificity phosphatase DSP-3, and polypeptide variants thereof that stimulate dephosphorylation of DSP-3 substrates, are provided. The polypeptides may be used, for example, to identify antibodies and other agents that inhibit DSP-3 activity. The polypeptides and agents may be used to modulate cell proliferation, differentiation and survival.

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## Description

### DSP-3 DUAL-SPECIFICITY PHOSPHATASE

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#### Technical Field

The present invention relates generally to compositions and methods useful for treating conditions associated with defects in cell proliferation, cell differentiation and/or cell survival. The invention is more particularly related to dual-specificity protein phosphatases, and polypeptide variants thereof. The present invention is also related to the use of such polypeptides to identify antibodies and other agents, including small molecules, that modulate signal transduction leading to proliferative responses, cell differentiation and/or cell survival.

#### 15 Background of the Invention

Mitogen-activated protein kinases (MAP-kinases) are present as components of conserved cellular signal transduction pathways that have a variety of conserved members. MAP-kinases are activated by phosphorylation at a dual phosphorylation motif with the sequence Thr-X-Tyr (by MAP-kinase kinases), in which phosphorylation at the tyrosine and threonine residues is required for activity. Activated MAP-kinases phosphorylate several transduction targets, including transcription factors. Inactivation of MAP-kinases is mediated by dephosphorylation at this site by dual-specificity phosphatases referred to as MAP-kinase phosphatases. In higher eukaryotes, the physiological role of MAP-kinase signaling has been correlated with cellular events such as proliferation, oncogenesis, development and differentiation. Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated with MAP-kinase signaling, such as cancer.

Dual-specificity protein tyrosine phosphatases (dual-specificity phosphatases) are phosphatases that dephosphorylate both phosphotyrosine and phosphothreonine/serine residues (Walton et al., *Ann. Rev. Biochem.* 62:101-120,

1993). Several dual-specificity phosphatases that inactivate a MAP-kinase have been identified, including MKP-1 (WO 97/00315; Keyse and Emslie, *Nature* 59:644-647, 1992), MKP-4, MKP-5, MKP-7, Hb5 (WO 97/06245), PAC1 (Ward et al., *Nature* 367:651-654, 1994), HVH2 (Guan and Butch, *J. Biol. Chem.* 270:7197-7203, 1995) and  
5 PYST1 (Groom et al., *EMBO J.* 15:3621-3632, 1996). Expression of certain dual-specificity phosphatases is induced by stress or mitogens, but others appear to be expressed constitutively in specific cell types. The regulation of dual-specificity phosphatase expression and activity is critical for control of MAP-kinase mediated cellular functions, including cell proliferation, cell differentiation and cell survival. For  
10 example, dual-specificity phosphatases may function as negative regulators of cell proliferation. It is likely that there are many such dual-specificity phosphatases, with varying specificity with regard to cell type or activation. However, the regulation of dual specificity phosphatases remains poorly understood and only a relatively small number of dual-specificity phosphatases have been identified.

15 Accordingly, there is a need in the art for an improved understanding of MAP-kinase signaling, and the regulation of dual-specificity phosphatases within MAP-kinase signaling cascades. An increased understanding of dual-specificity phosphatase regulation may facilitate the development of methods for modulating the activity of proteins involved in MAP-kinase cascades, and for treating conditions  
20 associated with such cascades. The present invention fulfills these needs and further provides other related advantages.

### Summary of the Invention

25 Briefly stated, the present invention provides compositions and methods for identifying agents capable of modulating cellular proliferative responses. In one aspect, the present invention provides isolated DSP-3 polypeptides having the sequence of DSP-3 recited in SEQ ID NO:2, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide retains the ability to dephosphorylate an  
30 activated MAP-kinase.

Within further aspects, the present invention provides an isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2. In certain embodiments the invention provides an isolated polynucleotide that encodes at least fifteen consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2. 5 Certain such polynucleotides encode a DSP-3 polypeptide. Still further, polynucleotides may be antisense polynucleotides that comprise at least 15 consecutive nucleotides complementary to a portion of a DSP-3 polynucleotide and/or that detectably hybridize to the complement of the sequence recited in SEQ ID NO:1 under 10 conditions that include a wash in 0.1X SSC and 0.1% SDS at 50°C for 15 minutes. Also provided are expression vectors comprising any of the foregoing polynucleotides, and host cells transformed or transfected with such expression vectors.

The present invention further provides, within other aspects, methods for producing a DSP-3 polypeptide, comprising the steps of: (a) culturing a host cell as 15 described above under conditions that permit expression of the DSP-3 polypeptide; and (b) isolating DSP-3 polypeptide from the host cell culture.

Also provided by the present invention are isolated antibodies, and antigen binding fragments thereof, that specifically bind to a DSP-3 polypeptide such as a polypeptide having the sequence of SEQ ID NO:2.

20 The present invention further provides, within other aspects, pharmaceutical compositions comprising a polypeptide, polynucleotide, antibody or fragment thereof as described above in combination with a physiologically acceptable carrier.

Within further aspects, the present invention provides methods for 25 detecting DSP-3 expression in a sample, comprising: (a) contacting a sample with an antibody or an antigen-binding fragment thereof as described above, under conditions and for a time sufficient to allow formation of an antibody/DSP-3 complex; and (b) detecting the level of antibody/DSP-3 complex.

Within still other aspects, the present invention provides methods for 30 detecting DSP-3 expression in a sample, comprising: (a) contacting a sample with an



antisense polynucleotide as described above; and (b) detecting in the sample an amount of DSP-3 polynucleotide that hybridizes to the antisense polynucleotide. The amount of DSP-3 polynucleotide that hybridizes to the antisense polynucleotide may be determined, for example, using polymerase chain reaction or a hybridization assay.

5           The invention also provides DSP-3 polypeptides useful in screening assays for modulators of enzyme activity and/or substrate binding. Methods are also provided, within other aspects, for screening for an agent that modulates DSP-3 activity, comprising the steps of: (a) contacting a candidate agent with a DSP-3 polypeptide as described above, under conditions and for a time sufficient to permit  
10 interaction between the polypeptide and candidate agent; and (b) subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-3 substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-3 substrate in the absence of candidate agent. Such methods may be performed *in vitro* or in a cellular environment (*e.g.*, within an intact cell).

15           Within further aspects, methods are provided for screening for an agent that modulates DSP-3 activity, comprising the steps of: (a) contacting a candidate agent with a cell comprising a DSP-3 promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and (b) subsequently evaluating  
20 the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent.

Also provided are methods for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-3 activity.

25           Within further aspects, methods are provided for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-3 activity.

The present invention further provides methods for modulating cell survival, comprising contacting a cell with an agent that modulates DSP-3 activity.

30           Within related aspects, the present invention provides methods for treating a patient afflicted with a disorder associated with DSP-3 activity (or treatable

by administration of DSP-3), comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-3 activity. Such disorders include Duchenne Muscular Dystrophy, as well as cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

Within further aspects, DSP-3 substrate trapping mutant polypeptides are provided. Such polypeptides differ from the sequence recited in SEQ ID NO:2 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide binds to a substrate with an affinity that is not substantially diminished relative to DSP-3, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-3. Within certain specific embodiments, a substrate trapping mutant polypeptide contains a substitution at position 57 or position 88 of SEQ ID NO:2.

The present invention further provides, within other aspects, methods for screening a molecule for the ability to interact with DSP-3, comprising the steps of: (a) contacting a candidate molecule with a polypeptide as described above under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and (b) detecting the presence or absence of binding of the candidate molecule to the polypeptide. The step of detecting may comprise, for example, an affinity purification step, a yeast two hybrid screen or a screen of a phage display library.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

#### Brief Description of the Drawings

Figure 1 presents a cDNA sequence for DSP-3 (SEQ ID NO:1), with the start and stop codons shown in bold.

Figure 2 presents the predicted amino acid sequence of DSP-3 (SEQ ID NO:2).

Figure 3 is a sequence alignment showing sequence similarity between DSP-3 and other MAP-kinase phosphatases.

Figure 4 shows northern blot hybridization using a  $^{32}\text{P}$ -labeled full length DSP-3 encoding nucleic acid sequence as probe. Blot contained human polyA+ RNA from various tissue types as follows: Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas.

#### Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for modulating (*i.e.*, stimulating or inhibiting) cellular proliferative responses, *in vitro* and *in vivo*. In particular, the present invention provides a dual-specificity phosphatase DSP-3 (Figures 1-2; SEQ ID NOs:1-2), as well as variants thereof and antibodies that specifically bind DSP-3. Also provided herein are methods for using such compounds for screens, detection assays and related therapeutic uses.

#### DSP-3 POLYPEPTIDES AND POLYNUCLEOTIDES

As used herein, the term "DSP-3 polypeptide" refers to a polypeptide that comprises a DSP-3 sequence as provided herein or a variant of such a sequence. Such polypeptides are capable of dephosphorylating both tyrosine and threonine/serine residues in a DSP-3 substrate, with an activity that is not substantially diminished relative to that of a full length native DSP-3. DSP-3 substrates include activated (*i.e.*, phosphorylated) MAP-kinases. Other substrates may be identified using substrate trapping mutants, as described herein, and include polypeptides having one or more phosphorylated tyrosine, threonine and /or serine residues.

DSP-3 polypeptide variants within the scope of the present invention may contain one or more substitutions, deletions, additions and/or insertions. For certain DSP-3 variants, the ability of the variant to dephosphorylate tyrosine and threonine residues within a DSP-3 substrate is not substantially diminished. The ability of such a DSP-3 variant to dephosphorylate tyrosine and threonine residues within a

DSP-3 substrate may be enhanced or unchanged, relative to a native DSP-3, or may be diminished by less than 50%, and preferably less than 20%, relative to native DSP-3. Such variants may be identified using the representative assays provided herein.

Also contemplated by the present invention are modified forms of DSP-3 in which a specific function is disabled. For example, such proteins may be constitutively active or inactive, or may display altered binding or catalytic properties. Such altered proteins may be generated using well known techniques, and the altered function confirmed using screens such as those provided herein. Certain modified DSP-3 polypeptides are known as "substrate trapping mutants." Such polypeptides retain the ability to bind a substrate (*i.e.*,  $K_m$  is not substantially diminished), but display a reduced ability to dephosphorylate a substrate (*i.e.*,  $k_{cat}$  is reduced, preferably to less than 1 per minute). Further, the stability of the substrate trapping mutant/substrate complex should not be substantially diminished, relative to the stability of a DSP-3/substrate complex. Complex stability may be assessed based on the association constant ( $K_a$ ). Determination of  $K_m$ ,  $k_{cat}$  and  $K_a$  may be readily accomplished using standard techniques known in the art (*see, e.g.*, WO 98/04712; Lehninger, Biochemistry, 1975 Worth Publishers, NY) and assays provided herein. Substrate trapping mutants may be generated, for example, by modifying DSP-3 with an amino acid substitution at position 57 or position 88 (*e.g.*, by replacing the amino acid aspartate at position 57 with an alanine residue, or by replacing the cysteine at residue 88 with a serine). Substrate trapping mutants may be used, for example, to identify DSP-3 substrates. Briefly, the modified DSP-3 may be contacted with a candidate substrate (alone or within a mixture of proteins, such as a cell extract) to permit the formation of a substrate/DSP-3 complex. The complex may then be isolated by conventional techniques to permit the isolation and characterization of substrate. The preparation and use of substrate trapping mutants is described, for example, within PCT Publication No. WO 98/04712.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide

chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes.

In general, modifications may be more readily made in non-critical regions, which are regions of the native sequence that do not substantially change the activity of DSP-3. Non-critical regions may be identified by modifying the DSP-3 sequence in a particular region and assaying the ability of the resulting variant in a phosphatase assay, as described herein. Preferred sequence modifications are made so as to retain the active site domain (VHCLAGVSR, SEQ ID NO:3). Within certain preferred embodiments, such modifications affect interactions between DSP-3 and cellular components other than DSP-3 substrates. However, substitutions may also be made in critical regions of the native protein, provided that the resulting variant substantially retains the ability to stimulate substrate dephosphorylation. Within certain embodiments, a variant contains substitutions, deletions, additions and/or insertions at no more than 50%, preferably no more than 25%, of the amino acid residues.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the polypeptide.

DSP-3 polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described below may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those having ordinary skill in the art.

5 Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells (including mammalian cells), and forms that differ in glycosylation may be generated by varying the host cell or post-isolation processing. Supernatants  
10 from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

15 Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic procedures, using techniques well known to those having ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method,  
20 where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin-Elmer, Inc., Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

25 A "DSP-3 polynucleotide" is any polynucleotide that encodes at least a portion of a DSP-3 polypeptide or a variant thereof, or that is complementary to such a polynucleotide. Preferred polynucleotides comprise at least 15 consecutive nucleotides, preferably at least 30 consecutive nucleotides, that encode a DSP-3 polypeptide or that are complementary to such a sequence. Certain polynucleotides encode a DSP-3  
30 polypeptide; others may find use as probes, primers or antisense oligonucleotides, as

described below. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be  
5 linked to other molecules and/or support materials.

DSP-3 polynucleotides may comprise a native sequence (*i.e.*, an endogenous DSP-3 sequence or a portion or splice variant thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the activity of the encoded  
10 polypeptide is not substantially diminished, as described above. The effect on the activity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native DSP-3 or a portion thereof. The percent identity may be  
15 readily determined by comparing sequences using computer algorithms well known to those having ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, *J. Mol. Biol.* 219:555-565, 1991; Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992), which is available at the NCBI website (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>). Default parameters may be used.  
20 Certain variants are substantially homologous to a native gene. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA or RNA sequence encoding a native DSP-3 (or a complementary sequence). Suitable moderately stringent conditions include, for example, prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-70°  
25 C, 5 X SSC, for 1-16 hours (*e.g.*, overnight); followed by washing once or twice at 22-65°C for 20-40 minutes with one or more each of 2X, 0.5X and 0.2X SSC containing 0.05-0.1% SDS. For additional stringency, conditions may include a wash in 0.1X SSC and 0.1% SDS at 50-60 °C for 15-40 minutes. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by  
30 altering the time, temperature and/or concentration of the solutions used for

prehybridization, hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a  
5 desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

It will also be appreciated by those having ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences  
10 that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

Polynucleotides may be prepared using any of a variety of techniques.  
15 For example, a polynucleotide may be amplified from cDNA prepared from a suitable cell or tissue type, such as human skeletal muscle cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion may be used to isolate a full length gene from a  
20 suitable library (*e.g.*, human skeletal muscle cell cDNA) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be  
25 preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by  
nick-translation or end-labeling with <sup>32</sup>P) using well known techniques. A bacterial or bacteriophage library may then be screened by hybridizing filters containing denatured  
30 bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see, e.g.*,



Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. Clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. One such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 17-32 nucleotides in length, have a GC content of at least 40% and anneal to the target sequence at temperatures of about 54°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

A cDNA sequence encoding DSP-3 is provided in Figure 1 (SEQ ID NO:1), and the predicted amino acid sequence is provided in Figure 2 (SEQ ID NO:2). The DSP-3 active site VHCLAGVSRS (SEQ ID NO:3), is encoded by nucleotide bases located at nucleotide positions 258 through 285 of SEQ ID NO:1 (Fig. 1; start codon begins at nucleotide position number 1). Sequence information immediately adjacent to this site was used to design 5' and 3' RACE reactions with human skeletal muscle cDNA to identify a protein of 184 amino acids encoded by 552 base pairs. This protein is referred to as dual specificity phosphatase-3, or DSP-3. Higher message abundance was observed for DSP-3 in human skeletal muscle tissue than in other tissues. DSP-3

shows significant homology to other MAP-kinase phosphatases, as shown by the sequence comparison presented in Figure 3.

DSP-3 polynucleotide variants may generally be prepared by any method known in the art, including, for example, solid phase chemical synthesis.

5 Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding DSP-3, or a portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or  
10 SP6). Certain polynucleotides may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a polynucleotide may be administered to a patient such that the encoded polypeptide is generated *in vivo*.

A polynucleotide that is complementary to at least a portion of a coding sequence (*e.g.*, an antisense polynucleotide or a ribozyme) may also be used as a probe  
15 or primer, or to modulate gene expression. Identification of oligonucleotides and ribozymes for use as antisense agents, and DNA encoding genes for their targeted delivery, involve methods well known in the art. For example, the desirable properties, lengths and other characteristics of such oligonucleotides are well known. Antisense oligonucleotides are typically designed to resist degradation by endogenous nucleolytic  
20 enzymes by using such linkages as: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages (*see, e.g.*, Agrwal et al., *Tetrahedron Lett.* 28:3539-3542 (1987); Miller et al., *J. Am. Chem. Soc.* 93:6657-6665 (1971); Stec et al., *Tetrahedron Lett.* 26:2191-2194 (1985); Moody et al., *Nucl. Acids Res.* 12:4769-4782 (1989); Uznanski et al., *Nucl.*  
25 *Acids Res.* (1989); Letsinger et al., *Tetrahedron* 40:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* 54:367-402 (1985); Eckstein, *Trends Biol. Sci.* 14:97-100 (1989); Stein In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp. 97-117 (1989); Jager et al., *Biochemistry* 27:7237-7246 (1988)).

Antisense polynucleotides are oligonucleotides that bind in a sequence-specific manner to nucleic acids, such as mRNA or DNA. When bound to mRNA that has complementary sequences, antisense prevents translation of the mRNA (*see, e.g.*, U.S. Patent No. 5,168,053 to Altman et al.; U.S. Patent No. 5,190,931 to Inouye, U.S. Patent No. 5,135,917 to Burch; U.S. Patent No. 5,087,617 to Smith and Clusel et al. (1993) *Nucl. Acids Res.* 21:3405-3411, which describes dumbbell antisense oligonucleotides). Triplex molecules refer to single DNA strands that bind duplex DNA forming a colinear triplex molecule, thereby preventing transcription (*see, e.g.*, U.S. Patent No. 5,176,996 to Hogan et al., which describes methods for making synthetic oligonucleotides that bind to target sites on duplex DNA).

Particularly useful antisense nucleotides and triplex molecules are molecules that are complementary to or bind the sense strand of DNA or mRNA that encodes a DSP-3 polypeptide or a protein mediating any other process related to expression of endogenous DSP-3, such that inhibition of translation of mRNA encoding the DSP-3 polypeptide is effected. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA. Antisense technology can be used to control gene expression through interference with binding of polymerases, transcription factors or other regulatory molecules (*see* Gee et al., *In* Huber and Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a DSP-3 gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

The present invention also contemplates DSP-3-specific ribozymes. A ribozyme is an RNA molecule that specifically cleaves RNA substrates, such as mRNA, resulting in specific inhibition or interference with cellular gene expression. There are at least five known classes of ribozymes involved in the cleavage and/or ligation of RNA chains. Ribozymes can be targeted to any RNA transcript and can catalytically cleave such transcripts (*see, e.g.*, U.S. Patent No. 5,272,262; U.S. Patent No. 5,144,019; and U.S. Patent Nos. 5,168,053, 5,180,818, 5,116,742 and 5,093,246 to

Cech et al.). Any DSP-3 mRNA-specific ribozyme, or a nucleic acid encoding such a ribozyme, may be delivered to a host cell to effect inhibition of DSP-3 gene expression. Ribozymes may therefore be delivered to the host cells by DNA encoding the ribozyme linked to a eukaryotic promoter, such as a eukaryotic viral promoter, such that upon  
5 introduction into the nucleus, the ribozyme will be directly transcribed.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages in the backbone; and/or the inclusion of nontraditional  
10 bases such as inosine, queosine and wybutosine, as well as acetyl-, methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of  
15 particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a suitable vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those having ordinary skill in the art.  
20

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those having ordinary skill in the art will appreciate that there are many ways to achieve expression of a  
25 polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector using well known techniques. A viral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target

cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those having ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

Within other aspects, a DSP-3 promoter may be isolated using standard techniques. The present invention provides nucleic acid molecules comprising such a promoter sequence or one or more cis- or trans-acting regulatory elements thereof. Such regulatory elements may enhance or suppress expression of DSP-3. A 5' flanking region may be generated using standard techniques, based on the genomic sequence provided herein. If necessary, additional 5' sequences may be generated using PCR-based or other standard methods. The 5' region may be subcloned and sequenced using standard methods. Primer extension and/or RNase protection analyses may be used to verify the transcriptional start site deduced from the cDNA.

To define the boundary of the promoter region, putative promoter inserts of varying sizes may be subcloned into a heterologous expression system containing a suitable reporter gene without a promoter or enhancer. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase or the Green Fluorescent Protein gene. Suitable expression systems are well known and may be prepared using well known techniques or obtained commercially. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of DSP-3 expression. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate DSP-3 transcription.

Once a functional promoter is identified, cis- and trans-acting elements may be located. Cis-acting sequences may generally be identified based on homology to previously characterized transcriptional motifs. Point mutations may then be generated within the identified sequences to evaluate the regulatory role of such sequences. Such mutations may be generated using site-specific mutagenesis techniques or a PCR-based strategy. The altered promoter is then cloned into a reporter gene expression vector, as described above, and the effect of the mutation on reporter gene expression is evaluated.

The present invention also contemplates the use of allelic variants of DSP-3, as well as DSP-3 sequences from other organisms. Such sequences may generally be identified based upon similarity to the sequences provided herein (*e.g.*, using hybridization techniques) and based upon the presence of DSP-3 activity, using an assay provided herein.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

#### ASSAYS FOR DETECTING DSP-3 ACTIVITY

According to the present invention, substrates of DSP-3 may include full length tyrosine phosphorylated proteins and polypeptides as well as fragments (*e.g.*, portions), derivatives or analogs thereof that can be phosphorylated at a tyrosine residue and that may, in certain preferred embodiments, also be able to undergo phosphorylation at a serine or a threonine residue. Such fragments, derivatives and analogs include any naturally occurring or artificially engineered DSP-3 substrate polypeptide that retains at least the biological function of interacting with a DSP-3 as provided herein, for example by forming a

complex with a DSP-3. A fragment, derivative or analog of a DSP-3 substrate polypeptide, including substrates that are fusion proteins, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), and such substituted amino acid residue may or  
5 may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the substrate polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (*e.g.*, polyethylene glycol) or a detectable moiety such as a reporter molecule, or (iv) one in  
10 which additional amino acids are fused to the substrate polypeptide, including amino acids that are employed for purification of the substrate polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art. In preferred embodiment, a MAP-kinase polypeptide is a substrate for use as provided herein.

DSP-3 polypeptide variants may be tested for DSP-3 activity using any  
15 suitable assay for MAP-kinase phosphatase activity. Such assays may be performed *in vitro* or within a cell-based assay. For example, a MAP-kinase may be obtained in inactive form from Upstate Biotechnology (Lake Placid, NY; catalog number 14-198), for use as a DSP-3 substrate as provided herein. Phosphorylation of the MAP-kinase can be performed using well known techniques (such as those described by Zheng and Guan, *J. Biol. Chem.*  
20 268:16116-16119, 1993) using the MAP-kinase kinase MEK-1 (available from Upstate Biotechnology; cat. no. 14-206).

For example, [<sup>32</sup>P]-radiolabeled substrate (*e.g.*, MAP-kinase) may be used for the kinase reaction, resulting in radiolabeled, activated MAP-kinase. A DSP-3 polypeptide may then be tested for the ability to dephosphorylate an activated MAP-  
25 kinase by contacting the DSP-3 polypeptide with the MAP-kinase under suitable conditions (*e.g.*, Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin for 10 minutes at 30°C; or as described by Zheng and Guan, *J. Biol. Chem.* 268:16116-16119, 1993). Dephosphorylation of the MAP-kinase may be detected using any of a variety of assays, such as a coupled kinase assay (evaluating  
30 phosphorylation of a MAP-kinase substrate using any assay generally known in the art)

or directly, based on (1) the loss of radioactive phosphate groups (e.g., by gel electrophoresis, followed by autoradiography); (2) the shift in electrophoretic mobility following dephosphorylation; (3) the loss of reactivity with an antibody specific for phosphotyrosine or phosphothreonine; or (4) a phosphoamino acid analysis of the  
5 MAP-kinase. Certain assays may generally be performed as described by Ward et al., *Nature* 367:651-654, 1994 or Alessi et al., *Oncogene* 8:2015-2020, 1993. In general, contact of 500 pg - 50 ng of DSP-3 polypeptide with 100ng - 100 µg activated MAP-kinase should result in a detectable dephosphorylation of the MAP-kinase, typically within 20-30 minutes. Within certain embodiments, 0.01 - 10 units/mL (preferably  
10 about 0.1 units/mL, where a unit is an amount sufficient to dephosphorylate 1 nmol substrate per minute) DSP-3 polypeptide may be contacted with 0.1 - 10 µM (preferably about 1 µM) activated MAP-kinase to produce a detectable dephosphorylation of a MAP-kinase. Preferably, a DSP-3 polypeptide results in a dephosphorylation of a MAP-kinase or a phosphorylated substrate (such as a tyrosine-  
15 and/or serine-phosphorylated peptide) that is at least as great as the dephosphorylation observed in the presence of a comparable amount of native human DSP-3. It will be apparent that other substrates identified using a substrate trapping mutant as described herein may be substituted for the MAP-kinase within such assays.

## 20 ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS

Also contemplated by the present invention are peptides, polypeptides, and other non-peptide molecules that specifically bind to a DSP-3. As used herein, a molecule is said to "specifically bind" to a DSP-3 if it reacts at a detectable level with DSP-3, but does not react detectably with peptides containing an unrelated sequence, or a sequence of a  
25 different phosphatase. Preferred binding molecules include antibodies, which may be, for example, polyclonal, monoclonal, single chain, chimeric, anti-idiotypic, or CDR-grafted immunoglobulins, or fragments thereof, such as proteolytically generated or recombinantly produced immunoglobulin F(ab')<sub>2</sub>, Fab, Fv, and Fd fragments. Certain preferred antibodies are those antibodies that inhibit or block DSP-3 activity within an *in vitro* assay, as described  
30 herein. Binding properties of an antibody to DSP-3 may generally be assessed using



immunodetection methods including, for example, an enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, immunoblotting and the like, which may be readily performed by those having ordinary skill in the art.

Methods well known in the art may be used to generate antibodies, polyclonal antisera or monoclonal antibodies that are specific for a DSP-3. Antibodies also may be produced as genetically engineered immunoglobulins (Ig) or Ig fragments designed to have desirable properties. For example, by way of illustration and not limitation, antibodies may include a recombinant IgG that is a chimeric fusion protein having at least one variable (V) region domain from a first mammalian species and at least one constant region domain from a second, distinct mammalian species. Most commonly, a chimeric antibody has murine variable region sequences and human constant region sequences. Such a murine/human chimeric immunoglobulin may be "humanized" by grafting the complementarity determining regions (CDRs) derived from a murine antibody, which confer binding specificity for an antigen, into human-derived V region framework regions and human-derived constant regions. Fragments of these molecules may be generated by proteolytic digestion, or optionally, by proteolytic digestion followed by mild reduction of disulfide bonds and alkylation. Alternatively, such fragments may also be generated by recombinant genetic engineering techniques.

As used herein, an antibody is said to be "immunospecific" or to "specifically bind" a DSP-3 polypeptide if it reacts at a detectable level with DSP-3, preferably with an affinity constant,  $K_a$ , of greater than or equal to about  $10^4 \text{ M}^{-1}$ , more preferably of greater than or equal to about  $10^5 \text{ M}^{-1}$ , more preferably of greater than or equal to about  $10^6 \text{ M}^{-1}$ , and still more preferably of greater than or equal to about  $10^7 \text{ M}^{-1}$ . Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example, those described by Scatchard et al. (*Ann. N.Y. Acad. Sci. USA* 51:660 (1949)) or by surface plasmon resonance (BIAcore, Biosensor, Piscataway, NJ). See, e.g., Wolff et al., *Cancer Res.* 53:2560-2565 (1993).

Antibodies may generally be prepared by any of a variety of techniques known to those having ordinary skill in the art. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988). In one such technique, an animal is immunized with DSP-3 as an antigen to generate polyclonal antisera. Suitable animals

include, for example, rabbits, sheep, goats, pigs, cattle, and may also include smaller mammalian species, such as mice, rats, and hamsters, or other species.

An immunogen may be comprised of cells expressing DSP-3, purified or partially purified DSP-3 polypeptides or variants or fragments (*e.g.*, peptides) thereof, or DSP-3 peptides. DSP-3 peptides may be generated by proteolytic cleavage or may be chemically synthesized. For instance, nucleic acid sequences encoding DSP-3 polypeptides are provided herein, such that those skilled in the art may routinely prepare these polypeptides for use as immunogens. Polypeptides or peptides useful for immunization may also be selected by analyzing the primary, secondary, and tertiary structure of DSP-3 according to methods known to those skilled in the art, in order to determine amino acid sequences more likely to generate an antigenic response in a host animal. *See, e.g.*, Novotny, 1991 *Mol. Immunol.* 28:201-207; Berzofsky, 1985 *Science* 229:932-40.

Preparation of the immunogen for injection into animals may include covalent coupling of the DSP-3 polypeptide (or variant or fragment thereof), to another immunogenic protein, for example, a carrier protein such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). In addition, the DSP-3 peptide, polypeptide, or DSP-3-expressing cells to be used as immunogen may be emulsified in an adjuvant. *See, e.g.*, Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988). In general, after the first injection, animals receive one or more booster immunizations according to a preferred schedule that may vary according to, *inter alia*, the antigen, the adjuvant (if any) and/or the particular animal species. The immune response may be monitored by periodically bleeding the animal, separating the sera out of the collected blood, and analyzing the sera in an immunoassay, such as an ELISA or Ouchterlony diffusion assay, or the like, to determine the specific antibody titer. Once an antibody titer is established, the animals may be bled periodically to accumulate the polyclonal antisera. Polyclonal antibodies that bind specifically to the DSP-3 polypeptide or peptide may then be purified from such antisera, for example, by affinity chromatography using protein A, or the DSP-3 polypeptide, immobilized on a suitable solid support.

Monoclonal antibodies that specifically bind to DSP-3 polypeptides or fragments or variants thereof, and hybridomas, which are immortal eukaryotic cell lines, that

produce monoclonal antibodies having the desired binding specificity, may also be prepared, for example, using the technique of Kohler and Milstein (*Nature*, 256:495-497; 1976, *Eur. J. Immunol.* 6:511-519 (1975)) and improvements thereto. An animal—for example, a rat, hamster, or preferably mouse—is immunized with a DSP-3 immunogen prepared as described above. Lymphoid cells that include antibody-forming cells, typically spleen cells, are obtained from an immunized animal and may be immortalized by fusion with a drug-sensitized myeloma (e.g., plasmacytoma) cell fusion partner, preferably one that is syngeneic with the immunized animal and that optionally has other desirable properties (e.g., inability to express endogenous Ig gene products). The lymphoid (e.g., spleen) cells and the myeloma cells may be combined for a few minutes with a membrane fusion-promoting agent, such as polyethylene glycol or a nonionic detergent, and then plated at low density on a selective medium that supports the growth of hybridoma cells, but not unfused myeloma cells. A preferred selection media is HAT (hypoxanthine, aminopterin, thymidine). After a sufficient time, usually about one to two weeks, colonies of cells are observed. Single colonies are isolated, and antibodies produced by the cells may be tested for binding activity to the DSP-3 polypeptide, or variant or fragment thereof. Hybridomas producing monoclonal antibodies with high affinity and specificity for a DSP-3 antigen are preferred. Hybridomas that produce monoclonal antibodies that specifically bind to a DSP-3 polypeptide or variant or fragment thereof are therefore contemplated by the present invention.

Monoclonal antibodies may be isolated from the supernatants of hybridoma cultures. An alternative method for production of a murine monoclonal antibody is to inject the hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (e.g., pristane-primed) to promote formation of ascites fluid containing the monoclonal antibody. Contaminants may be removed from the subsequently (usually within 1-3 weeks) harvested ascites fluid by conventional techniques, such as chromatography, gel filtration, precipitation, extraction, or the like. For example, antibodies may be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the monoclonal antibody (e.g., heavy or light chain isotype, binding specificity, etc.). Examples of a suitable ligand, immobilized on a solid support, include

Protein A, Protein G, an anti-constant region (light chain or heavy chain) antibody, an anti-idiotypic antibody and a DSP-3 polypeptide or fragment or variant thereof.

Human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such methods include but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (e.g., containing B lymphocytes), *in vitro* immunization of human B cells, fusion of spleen cells from immunized transgenic mice carrying human immunoglobulin genes inserted by yeast artificial chromosomes (YAC), isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein.

For example, one method for generating human monoclonal antibodies includes immortalizing human peripheral blood cells by EBV transformation. *See, e.g.*, U.S. Patent No. 4,464,456. An immortalized cell line producing a monoclonal antibody that specifically binds to a DSP-3 polypeptide (or a variant or fragment thereof) can be identified by immunodetection methods as provided herein, for example, an ELISA, and then isolated by standard cloning techniques. Another method to generate human monoclonal antibodies, *in vitro* immunization, includes priming human splenic B cells with antigen, followed by fusion of primed B cells with a heterohybrid fusion partner. *See, e.g.*, Boerner et al., 1991 *J. Immunol.* 147:86-95.

Still another method for the generation of human DSP-3-specific monoclonal antibodies and polyclonal antisera for use in the present invention relates to transgenic mice. *See, e.g.*, U.S. Patent No. 5,877,397; Bruggemann et al., 1997 *Curr. Opin. Biotechnol.* 8:455-58; Jakobovits et al., 1995 *Ann. N. Y. Acad. Sci.* 764:525-35. In these mice, human immunoglobulin heavy and light chain genes have been artificially introduced by genetic engineering in germline configuration, and the endogenous murine immunoglobulin genes have been inactivated. *See, e.g.*, Bruggemann et al., 1997 *Curr. Opin. Biotechnol.* 8:455-58. For example, human immunoglobulin transgenes may be mini-gene constructs, or translocated on yeast artificial chromosomes, which undergo B cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue. *See, Bruggemann et al., 1997 Curr. Opin. Biotechnol.* 8:455-58. Human monoclonal antibodies specifically binding to DSP-3 may be obtained by immunizing the transgenic animals, fusing spleen cells with myeloma cells,

selecting and then cloning cells producing antibody, as described above. Polyclonal sera containing human antibodies may also be obtained from the blood of the immunized animals.

Chimeric antibodies, specific for a DSP-3, including humanized antibodies, may also be generated according to the present invention. A chimeric antibody has at least one constant region domain derived from a first mammalian species and at least one variable region domain derived from a second, distinct mammalian species. *See, e.g., Morrison et al., 1984, Proc. Natl. Acad. Sci. USA, 81:6851-55.* In preferred embodiments, a chimeric antibody may be constructed by cloning the polynucleotide sequence that encodes at least one variable region domain derived from a non-human monoclonal antibody, such as the variable region derived from a murine, rat, or hamster monoclonal antibody, into a vector containing a nucleic acid sequence that encodes at least one human constant region. *See, e.g., Shin et al., 1989 Methods Enzymol. 178:459-76; Walls et al., 1993 Nucleic Acids Res. 21:2921-29.* By way of example, the polynucleotide sequence encoding the light chain variable region of a murine monoclonal antibody may be inserted into a vector containing a nucleic acid sequence encoding the human kappa light chain constant region sequence. In a separate vector, the polynucleotide sequence encoding the heavy chain variable region of the monoclonal antibody may be cloned in frame with sequences encoding the human IgG1 constant region. The particular human constant region selected may depend upon the effector functions desired for the particular antibody (*e.g., complement fixing, binding to a particular Fc receptor, etc.*). Another method known in the art for generating chimeric antibodies is homologous recombination (*e.g., U.S. Patent No. 5,482,856*). Preferably, the vectors will be transfected into eukaryotic cells for stable expression of the chimeric antibody.

A non-human/human chimeric antibody may be further genetically engineered to create a "humanized" antibody. Such a humanized antibody may comprise a plurality of CDRs derived from an immunoglobulin of a non-human mammalian species, at least one human variable framework region, and at least one human immunoglobulin constant region. Humanization may in certain embodiments provide an antibody that has decreased binding affinity for a DSP-3 when compared, for example, with either a non-human monoclonal antibody from which a DSP-3 binding variable region is obtained, or a chimeric antibody having such a V region and at least one human C region, as described above. Useful

strategies for designing humanized antibodies may therefore include, for example by way of illustration and not limitation, identification of human variable framework regions that are most homologous to the non-human framework regions of the chimeric antibody. Without wishing to be bound by theory, such a strategy may increase the likelihood that the humanized antibody will retain specific binding affinity for a DSP-3, which in some preferred embodiments may be substantially the same affinity for a DSP-3 polypeptide or variant or fragment thereof, and in certain other preferred embodiments may be a greater affinity for DSP-3. See, e.g., Jones et al., 1986 *Nature* 321:522-25; Riechmann et al., 1988 *Nature* 332:323-27. Designing such a humanized antibody may therefore include determining CDR loop conformations and structural determinants of the non-human variable regions, for example, by computer modeling, and then comparing the CDR loops and determinants to known human CDR loop structures and determinants. See, e.g., Padlan et al., 1995 *FASEB* 9:133-39; Chothia et al., 1989 *Nature*, 342:377-383. Computer modeling may also be used to compare human structural templates selected by sequence homology with the non-human variable regions. See, e.g., Bajorath et al., 1995 *Ther. Immunol.* 2:95-103; EP-0578515-A3. If humanization of the non-human CDRs results in a decrease in binding affinity, computer modeling may aid in identifying specific amino acid residues that could be changed by site-directed or other mutagenesis techniques to partially, completely or supra-optimally (*i.e.*, increase to a level greater than that of the non-humanized antibody) restore affinity. Those having ordinary skill in the art are familiar with these techniques, and will readily appreciate numerous variations and modifications to such design strategies.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments or F(ab')<sub>2</sub> fragments, which may be prepared by proteolytic digestion with papain or pepsin, respectively. The antigen binding fragments may be separated from the Fc fragments by affinity chromatography, for example, using immobilized protein A or protein G, or immobilized DSP-3 polypeptide, or a suitable variant or fragment thereof. Those having ordinary skill in the art can routinely and without undue experimentation determine what is a suitable variant or fragment based on characterization of affinity purified antibodies obtained, for example, using immunodetection methods as provided herein. An alternative method to generate Fab

fragments includes mild reduction of  $F(ab')_2$  fragments followed by alkylation. *See, e.g., Weir, Handbook of Experimental Immunology*, 1986, Blackwell Scientific, Boston.

According to certain embodiments, non-human, human, or humanized heavy chain and light chain variable regions of any of the above described Ig molecules may be constructed as single chain Fv (sFv) polypeptide fragments (single chain antibodies). *See, e.g., Bird et al., 1988 Science 242:423-426; Huston et al., 1988 Proc. Natl. Acad. Sci. USA 85:5879-5883.* Multi-functional sFv fusion proteins may be generated by linking a polynucleotide sequence encoding an sFv polypeptide in-frame with at least one polynucleotide sequence encoding any of a variety of known effector proteins. These methods are known in the art, and are disclosed, for example, in EP-B1-0318554, U.S. Patent No. 5,132,405, U.S. Patent No. 5,091,513, and U.S. Patent No. 5,476,786. By way of example, effector proteins may include immunoglobulin constant region sequences. *See, e.g., Hollenbaugh et al., 1995 J. Immunol. Methods 188:1-7.* Other examples of effector proteins are enzymes. As a non-limiting example, such an enzyme may provide a biological activity for therapeutic purposes (*see, e.g., Siemers et al., 1997 Bioconjug. Chem. 8:510-19*), or may provide a detectable activity, such as horseradish peroxidase-catalyzed conversion of any of a number of well-known substrates into a detectable product, for diagnostic uses. Still other examples of sFv fusion proteins include Ig-toxin fusions, or immunotoxins, wherein the sFv polypeptide is linked to a toxin. Those having ordinary skill in the art will appreciate that a wide variety of polypeptide sequences have been identified that, under appropriate conditions, are toxic to cells. As used herein, a toxin polypeptide for inclusion in an immunoglobulin-toxin fusion protein may be any polypeptide capable of being introduced to a cell in a manner that compromises cell survival, for example, by directly interfering with a vital function or by inducing apoptosis. Toxins thus may include, for example, ribosome-inactivating proteins, such as *Pseudomonas aeruginosa* exotoxin A, plant gelonin, bryodin from *Bryonia dioica*, or the like. *See, e.g., Thrush et al., 1996 Annu. Rev. Immunol. 14:49-71; Frankel et al., 1996 Cancer Res. 56:926-32.* Numerous other toxins, including chemotherapeutic agents, anti-mitotic agents, antibiotics, inducers of apoptosis (or "apoptogens", *see, e.g., Green and Reed, 1998, Science 281:1309-1312*), or the like, are known to those familiar with the art, and the

examples provided herein are intended to be illustrative without limiting the scope and spirit of the invention.

The sFv may, in certain embodiments, be fused to peptide or polypeptide domains that permit detection of specific binding between the fusion protein and antigen (e.g., a DSP-3). For example, the fusion polypeptide domain may be an affinity tag polypeptide. Binding of the sFv fusion protein to a binding partner (e.g., a DSP-3) may therefore be detected using an affinity polypeptide or peptide tag, such as an avidin, streptavidin or a His (e.g., polyhistidine) tag, by any of a variety of techniques with which those skilled in the art will be familiar. Detection techniques may also include, for example, binding of an avidin or streptavidin fusion protein to biotin or to a biotin mimetic sequence (see, e.g., Luo et al., 1998 *J. Biotechnol.* 65:225 and references cited therein), direct covalent modification of a fusion protein with a detectable moiety (e.g., a labeling moiety), non-covalent binding of the fusion protein to a specific labeled reporter molecule, enzymatic modification of a detectable substrate by a fusion protein that includes a portion having enzyme activity, or immobilization (covalent or non-covalent) of the fusion protein on a solid-phase support.

The sFv fusion protein of the present invention, comprising a DSP-3-specific immunoglobulin-derived polypeptide fused to another polypeptide such as an effector peptide having desirable affinity properties, may therefore include, for example, a fusion protein wherein the effector peptide is an enzyme such as glutathione-S-transferase. As another example, sFv fusion proteins may also comprise a DSP-3-specific Ig polypeptide fused to a *Staphylococcus aureus* protein A polypeptide; protein A encoding nucleic acids and their use in constructing fusion proteins having affinity for immunoglobulin constant regions are disclosed generally, for example, in U.S. Patent 5,100,788. Other useful affinity polypeptides for construction of sFv fusion proteins may include streptavidin fusion proteins, as disclosed, for example, in WO 89/03422; U.S. 5,489,528; U.S. 5,672,691; WO 93/24631; U.S. 5,168,049; U.S. 5,272,254 and elsewhere, and avidin fusion proteins (see, e.g., EP 511,747). As provided herein, sFv polypeptide sequences may be fused to fusion polypeptide sequences, including effector protein sequences, that may include full length fusion polypeptides and that may alternatively contain variants or fragments thereof.



An additional method for selecting antibodies that specifically bind to a DSP-3 polypeptide or variant or fragment thereof is by phage display. *See, e.g.,* Winter et al., 1994 *Annul. Rev. Immunol.* 12:433-55; Burton et al., 1994 *Adv. Immunol.* 57:191-280. Human or murine immunoglobulin variable region gene combinatorial libraries may be created in  
5 phage vectors that can be screened to select Ig fragments (Fab, Fv, sFv, or multimers thereof) that bind specifically to a DSP-3 polypeptide or variant or fragment thereof. *See, e.g.,* U.S. Patent No. 5,223,409; Huse et al., 1989 *Science* 246:1275-81; Kang et al., 1991 *Proc. Natl. Acad. Sci. USA* 88:4363-66; Hoogenboom et al., 1992 *J. Molec. Biol.* 227:381-388; Schlebusch et al., 1997 *Hybridoma* 16:47-52 and references cited therein. For example, a  
10 library containing a plurality of polynucleotide sequences encoding Ig variable region fragments may be inserted into the genome of a filamentous bacteriophage, such as M13 or a variant thereof, in frame with the sequence encoding a phage coat protein, for instance, gene III or gene VIII of M13, to create an M13 fusion protein. A fusion protein may be a fusion of the coat protein with the light chain variable region domain and/or with the heavy chain  
15 variable region domain.

According to certain embodiments, immunoglobulin Fab fragments may also be displayed on the phage particle, as follows. Polynucleotide sequences encoding Ig constant region domains may be inserted into the phage genome in frame with a coat protein. The phage coat fusion protein may thus be fused to an Ig light chain or heavy chain fragment  
20 (Fd). For example, from a human Ig library, the polynucleotide sequence encoding the human kappa constant region may be inserted into a vector in frame with the sequence encoding at least one of the phage coat proteins. Additionally or alternatively, the polynucleotide sequence encoding the human IgG1 CH1 domain may be inserted in frame with the sequence encoding at least one other of the phage coat proteins. A plurality of  
25 polynucleotide sequences encoding variable region domains (*e.g.,* derived from a DNA library) may then be inserted into the vector in frame with the constant region-coat protein fusions, for expression of Fab fragments fused to a bacteriophage coat protein.

Phage that display an Ig fragment (*e.g.,* an Ig V-region or Fab) that binds to a DSP-3 polypeptide may be selected by mixing the phage library with DSP-3 or a variant or a  
30 fragment thereof, or by contacting the phage library with a DSP-3 polypeptide immobilized

on a solid matrix under conditions and for a time sufficient to allow binding. Unbound phage are removed by a wash, which typically may be a buffer containing salt (e.g., NaCl) at a low concentration, preferably with less than 100 mM NaCl, more preferably with less than 50 mM NaCl, most preferably with less than 10 mM NaCl, or, alternatively, a buffer containing no salt. Specifically bound phage are then eluted with an NaCl-containing buffer, for example, by increasing the salt concentration in a step-wise manner. Typically, phage that bind the DSP-3 with higher affinity will require higher salt concentrations to be released. Eluted phage may be propagated in an appropriate bacterial host, and generally, successive rounds of DSP-3 binding and elution can be repeated to increase the yield of phage expressing DSP-3 specific immunoglobulin. Combinatorial phage libraries may also be used for humanization of non-human variable regions. See, e.g., Rosok et al., 1996 *J. Biol. Chem.* 271:22611-18; Rader et al., 1998 *Proc. Natl. Acad. Sci. USA* 95:8910-15. The DNA sequence of the inserted immunoglobulin gene in the phage so selected may be determined by standard techniques. See, Sambrook et al., 1989 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press. The affinity selected Ig-encoding sequence may then be cloned into another suitable vector for expression of the Ig fragment or, optionally, may be cloned into a vector containing Ig constant regions, for expression of whole immunoglobulin chains.

Phage display techniques may also be used to select polypeptides, peptides or single chain antibodies that bind to DSP-3. For examples of suitable vectors having multicloning sites into which candidate nucleic acid molecules (e.g., DNA) encoding such peptides or antibodies may be inserted, see, e.g., McLafferty et al., *Gene* 128:29-36, 1993; Scott et al., 1990 *Science* 249:386-390; Smith et al., 1993 *Methods Enzymol.* 217:228-257; Fisch et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:7761-66. The inserted DNA molecules may comprise randomly generated sequences, or may encode variants of a known peptide or polypeptide domain that specifically binds to a DSP-3 polypeptide, or variant or fragment thereof, as provided herein. Generally, the nucleic acid insert encodes a peptide of up to 60 amino acids, more preferably a peptide of 3 to 35 amino acids, and still more preferably a peptide of 6 to 20 amino acids. The peptide encoded by the inserted sequence is displayed on the surface of the bacteriophage. Phage expressing a binding domain for a DSP-3 polypeptide may be selected on the basis of specific binding to an immobilized DSP-3

polypeptide as described above. As provided herein, well-known recombinant genetic techniques may be used to construct fusion proteins containing the fragment thereof. For example, a polypeptide may be generated that comprises a tandem array of two or more similar or dissimilar affinity selected DSP-3 binding peptide domains, in order to maximize binding affinity for DSP-3 of the resulting product.

In certain other embodiments, the invention contemplates DSP-3 specific antibodies that are multimeric antibody fragments. Useful methodologies are described generally, for example in Hayden et al. 1997, *Curr Opin. Immunol.* 9:201-12; Coloma et al., 1997 *Nat. Biotechnol.* 15:159-63). For example, multimeric antibody fragments may be created by phage techniques to form miniantibodies (U.S. Patent No. 5,910 573) or diabodies (Holliger et al., 1997, *Cancer Immunol. Immunother.* 45:128-130). Multimeric fragments may be generated that are multimers of a DSP-3-specific Fv, or that are bispecific antibodies comprising a DSP-3-specific Fv noncovalently associated with a second Fv having a different antigen specificity. See, e.g., Koelemij et al., 1999 *J. Immunother.* 22:514-24. As another example, a multimeric antibody may comprise a bispecific antibody having two single chain antibodies or Fab fragments. According to certain related embodiments, a first Ig fragment may be specific for a first antigenic determinant on a DSP-3 polypeptide (or variant or fragment thereof), while a second Ig fragment may be specific for a second antigenic determinant of the DSP-3 polypeptide. Alternatively, in certain other related embodiments, a first immunoglobulin fragment may be specific for an antigenic determinant on a DSP-3 polypeptide or variant or fragment thereof, and a second immunoglobulin fragment may be specific for an antigenic determinant on a second, distinct (i.e., non-DSP-3) molecule. Also contemplated are bispecific antibodies that specifically bind DSP-3, wherein at least one antigen-binding domain is present as a fusion protein.

Introducing amino acid mutations into DSP-3-binding immunoglobulin molecules may be useful to increase the specificity or affinity for DSP-3, or to alter an effector function. Immunoglobulins with higher affinity for DSP-3 may be generated by site-directed mutagenesis of particular residues. Computer assisted three-dimensional molecular modeling may be employed to identify the amino acid residues to be changed, in order to improve affinity for the DSP-3 polypeptide. See, e.g., Mountain et al., 1992, *Biotechnol.*

*Genet. Eng. Rev.* 10: 1-142. Alternatively, combinatorial libraries of CDRs may be generated in M13 phage and screened for immunoglobulin fragments with improved affinity. See, e.g., Glaser et al., 1992, *J. Immunol.* 149:3903-3913; Barbas et al., 1994 *Proc. Natl. Acad. Sci. USA* 91:3809-13; U.S. Patent No. 5,792, 456).

5           Effector functions may also be altered by site-directed mutagenesis. See, e.g., Duncan et al., 1988 *Nature* 332:563-64; Morgan et al., 1995 *Immunology* 86:319-24; Eghtedarzede-Kondri et al., 1997 *Biotechniques* 23:830-34. For example, mutation of the glycosylation site on the Fc portion of the immunoglobulin may alter the ability of the immunoglobulin to fix complement. See, e.g., Wright et al., 1997 *Trends Biotechnol.* 15:26-  
10 32. Other mutations in the constant region domains may alter the ability of the immunoglobulin to fix complement, or to effect antibody-dependent cellular cytotoxicity. See, e.g., Duncan et al., 1988 *Nature* 332:563-64; Morgan et al., 1995 *Immunology* 86:319-24; Sensel et al., 1997 *Mol. Immunol.* 34:1019-29.

          The nucleic acid molecules encoding an antibody or fragment thereof that  
15 specifically binds DSP-3, as described herein, may be propagated and expressed according to any of a variety of well-known procedures for nucleic acid excision, ligation, transformation and transfection. Thus, in certain embodiments expression of an antibody fragment may be preferred in a prokaryotic host, such as *Escherichia coli* (see, e.g., Pluckthun et al., 1989 *Methods Enzymol.* 178:497-515). In certain other embodiments, expression of the antibody  
20 or a fragment thereof may be preferred in a eukaryotic host cell, including yeast (e.g., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*), animal cells (including mammalian cells) or plant cells. Examples of suitable animal cells include, but are not limited to, myeloma, COS, CHO, or hybridoma cells. Examples of plant cells include tobacco, corn, soybean, and rice cells. By methods known to those having ordinary skill in  
25 the art and based on the present disclosure, a nucleic acid vector may be designed for expressing foreign sequences in a particular host system, and then polynucleotide sequences encoding the DSP-3 binding antibody (or fragment thereof) may be inserted. The regulatory elements will vary according to the particular host.

          A DSP-3-binding immunoglobulin (or fragment thereof) as described herein  
30 may contain a detectable moiety or label such as an enzyme, cytotoxic agent or other reporter

molecule, including a dye, radionuclide, luminescent group, fluorescent group, or biotin, or the like. The DSP-3-specific immunoglobulin or fragment thereof may be radiolabeled for diagnostic or therapeutic applications. Techniques for radiolabeling of antibodies are known in the art. *See, e.g.,* Adams 1998 *In Vivo* 12:11-21; Hiltunen 1993 *Acta Oncol.* 32:831-9.

5 Therapeutic applications are described in greater detail below and may include use of the DSP-3-binding antibody (or fragment thereof) in conjunction with other therapeutic agents. The antibody or fragment may also be conjugated to a cytotoxic agent as known in the art and provided herein, for example, a toxin, such as a ribosome-inactivating protein, a chemotherapeutic agent, an anti-mitotic agent, an antibiotic or the like.

10 The invention also contemplates the generation of anti-idiotypic antibodies that recognize an antibody (or antigen-binding fragment thereof) that specifically binds to DSP-3 as provided herein, or a variant or fragment thereof. Anti-idiotypic antibodies may be generated as polyclonal antibodies or as monoclonal antibodies by the methods described herein, using an anti-DSP-3 antibody (or antigen-binding fragment thereof) as immunogen.

15 Anti-idiotypic antibodies or fragments thereof may also be generated by any of the recombinant genetic engineering methods described above, or by phage display selection. An anti-idiotypic antibody may react with the antigen binding site of the anti-DSP-3 antibody such that binding of the anti-DSP-3 antibody to a DSP-3 polypeptide is competitively inhibited. Alternatively, an anti-idiotypic antibody as provided herein may not competitively

20 inhibit binding of an anti-DSP-3 antibody to a DSP-3 polypeptide.

As provided herein and according to methodologies well known in the art, polyclonal and monoclonal antibodies may be used for the affinity isolation of DSP-3 polypeptides. *See, e.g.,* Hermanson et al., *Immobilized Affinity Ligand Techniques*, Academic Press, Inc. New York, 1992. Briefly, an antibody (or antigen-binding fragment thereof) may

25 be immobilized on a solid support material, which is then contacted with a sample comprising the polypeptide of interest (*e.g.,* a DSP-3). Following separation from the remainder of the sample, the polypeptide is then released from the immobilized antibody.

#### METHODS FOR DETECTING DSP-3 EXPRESSION

30 Certain aspects of the present invention provide methods that employ antibodies raised against DSP-3, or hybridizing polynucleotides, for diagnostic and

assay purposes. Certain assays involve using an antibody or other agent to detect the presence or absence of DSP-3, or proteolytic fragments thereof. Alternatively, nucleic acid encoding DSP-3 may be detected, using standard hybridization and/or PCR techniques. Suitable probes and primers may be designed by those having ordinary skill in the art based on the DSP-3 cDNA sequence provided herein. Assays may generally be performed using any of a variety of samples obtained from a biological source, such as eukaryotic cells, bacteria, viruses, extracts prepared from such organisms and fluids found within living organisms. Biological samples that may be obtained from a patient include blood samples, biopsy specimens, tissue explants, organ cultures and other tissue or cell preparations. A patient or biological source may be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiable cell lines, transformed cell lines and the like. In certain preferred embodiments the patient or biological source is a human, and in certain preferred embodiments the biological source is a non-human animal that is a mammal, for example, a rodent (*e.g.*, mouse, rat, hamster, etc.), an ungulate (*e.g.*, bovine) or a non-human primate. In certain other preferred embodiments of the invention, a patient may be suspected of having or being at risk for having a disease associated with altered cellular signal transduction, or may be known to be free of a risk for or presence of such as disease.

To detect DSP-3 protein, the reagent is typically an antibody, which may be prepared as described below. There are a variety of assay formats known to those having ordinary skill in the art for using an antibody to detect a polypeptide in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, the assay may be performed in a Western blot format, wherein a protein preparation from the biological sample is resolved by gel electrophoresis, transferred to a suitable membrane and allowed to react with the

antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as described below.

In another embodiment, the assay involves the use of antibody immobilized on a solid support to bind to the target DSP-3 and remove it from the remainder of the sample. The bound DSP-3 may then be detected using a second antibody or reagent that contains a reporter group. Alternatively, a competitive assay may be utilized, in which a DSP-3 polypeptide is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the level of DSP-3 in the sample.

The solid support may be any material known to those having ordinary skill in the art to which the antibody may be attached, such as a test well in a microtiter plate, a nitrocellulose filter or another suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic such as polystyrene or polyvinylchloride. The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature.

In certain embodiments, the assay for detection of DSP-3 in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that DSP-3 within the sample is allowed to bind to the immobilized antibody (a 30 minute incubation time at room temperature is generally sufficient). Unbound sample is then removed from the immobilized DSP-3/antibody complexes and a second antibody (containing a reporter group such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin) capable of binding to a different site on the DSP-3 is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic

methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time),  
5 followed by spectroscopic or other analysis of the reaction products. Standards and standard additions may be used to determine the level of DSP-3 in a sample, using well known techniques.

In a related aspect of the present invention, kits for detecting DSP-3 and DSP-3 phosphatase activity are provided. Such kits may be designed for detecting the  
10 level of DSP-3 or nucleic acid encoding DSP-3, or may detect phosphatase activity of DSP-3 in a direct phosphatase assay or a coupled phosphatase assay. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay.

A kit for detecting the level of DSP-3, or nucleic acid encoding DSP-3,  
15 typically contains a reagent that binds to the DSP-3 protein, DNA or RNA. To detect nucleic acid encoding DSP-3, the reagent may be a nucleic acid probe or a PCR primer. To detect DSP-3 protein, the reagent is typically an antibody. Such kits also contain a reporter group suitable for direct or indirect detection of the reagent (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule,  
20 such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample  
25 component using standard methods known to those having ordinary skill in the art.

Kits for detecting DSP-3 activity typically comprise a DSP-3 substrate in combination with a suitable buffer. DSP-3 activity may be specifically detected by performing an immunoprecipitation step with a DSP-3-specific antibody prior to performing a phosphatase assay as described above. Other reagents for use in detecting  
30 dephosphorylation of substrate may also be provided.



Within certain diagnostic assays, a proliferative disorder may be detected in a patient or any other biological source organism as provided herein based on the presence of an altered DSP-3 or an altered level of DSP-3 expression. For example, an antibody may distinguish between a wild-type DSP-3 and an altered DSP-3 having a variation in amino acid sequence. Such a variation may be indicative of the presence of a proliferative disorder, or of susceptibility to such a disorder. Hybridization and amplification techniques may be similarly used to detect modified DSP-3 sequences.

#### 10 METHODS FOR IDENTIFYING MODULATORS OF DSP-3 ACTIVITY

In one aspect of the present invention, DSP-3 polypeptides may be used to identify agents that modulate DSP-3 activity. Such agents may inhibit or enhance signal transduction via a MAP-kinase cascade, leading to cell proliferation. An agent that modulates DSP-3 activity may alter expression and/or stability of DSP-3, DSP-3 protein activity and/or the ability of DSP-3 to dephosphorylate a substrate. Agents that may be screened within such assays include, but are not limited to, antibodies and antigen-binding fragments thereof, competing substrates or peptides that represent, for example, a catalytic site or a dual phosphorylation motif, antisense polynucleotides and ribozymes that interfere with transcription and/or translation of DSP-3 and other natural and synthetic molecules, for example small molecule inhibitors, that bind to and inactivate DSP-3.

Candidate agents for use in a method of screening for a modulator of DSP-3 according to the present invention may be provided as "libraries" or collections of compounds, compositions or molecules. Such molecules typically include compounds known in the art as "small molecules" and having molecular weights less than  $10^5$  daltons, preferably less than  $10^4$  daltons and still more preferably less than  $10^3$  daltons. For example, members of a library of test compounds can be administered to a plurality of samples, each containing at least one DSP-3 polypeptide as provided herein, and then assayed for their ability to enhance or inhibit DSP-3-mediated dephosphorylation of, or binding to, a substrate. Compounds so identified as capable of

influencing DSP-3 function (*e.g.*, phosphotyrosine and/or phosphoserine/threonine dephosphorylation) are valuable for therapeutic and/or diagnostic purposes, since they permit treatment and/or detection of diseases associated with DSP-3 activity. Such compounds are also valuable in research directed to molecular signaling mechanisms that involve DSP-3, and to refinements in the discovery and development of future DSP-3 compounds exhibiting greater specificity.

Candidate agents further may be provided as members of a combinatorial library, which preferably includes synthetic agents prepared according to a plurality of predetermined chemical reactions performed in a plurality of reaction vessels. For example, various starting compounds may be prepared employing one or more of solid-phase synthesis, recorded random mix methodologies and recorded reaction split techniques that permit a given constituent to traceably undergo a plurality of permutations and/or combinations of reaction conditions. The resulting products comprise a library that can be screened followed by iterative selection and synthesis procedures, such as a synthetic combinatorial library of peptides (see *e.g.*, PCT/US91/08694, PCT/US91/04666, which are hereby incorporated by reference in their entireties) or other compositions that may include small molecules as provided herein (see *e.g.*, PCT/US94/08542, EP 0774464, U.S. 5,798,035, U.S. 5,789,172, U.S. 5,751,629, which are hereby incorporated by reference in their entireties). Those having ordinary skill in the art will appreciate that a diverse assortment of such libraries may be prepared according to established procedures, and tested using DSP-3 according to the present disclosure.

In certain embodiments, modulating agents may be identified by combining a candidate agent with a DSP-3 polypeptide or a polynucleotide encoding such a polypeptide, *in vitro* or *in vivo*, and evaluating the effect of the candidate agent on the DSP-3 phosphatase activity using, for example, a representative assay described herein. An increase or decrease in phosphatase activity can be measured by performing a representative assay provided herein in the presence and absence of a candidate agent. Briefly, a candidate agent may be included in a mixture of active DSP-3 polypeptide and substrate (*e.g.*, a phosphorylated MAP-kinase), with or without pre-incubation with

one or more components of the mixture. In general, a suitable amount of antibody or other agent for use in such an assay ranges from about 0.01  $\mu$ M to about 100  $\mu$ M. The effect of the agent on DSP-3 activity may then be evaluated by quantifying the loss of phosphate from the substrate, and comparing the loss with that achieved using DSP-3 without the addition of a candidate agent. Alternatively, a coupled kinase assay may be used, in which DSP-3 activity is indirectly measured based on MAP-kinase activity.

Alternatively, a polynucleotide comprising a DSP-3 promoter operably linked to a DSP-3 coding region or reporter gene may be used to evaluate the effect of a test compound on DSP-3 transcription. Such assays may be performed in cells that express DSP-3 endogenously (*e.g.*, human or other mammalian skeletal muscle, heart, brain, liver or pancreatic cells) or in cells transfected with an expression vector comprising a DSP-3 promoter linked to a reporter gene. The effect of a test compound may then be evaluated by assaying the effect on transcription of DSP-3 or the reporter using, for example, a Northern blot analysis or a suitable reporter activity assay.

DSP-3 activity may also be measured in whole cells transfected with a reporter gene whose expression is dependent upon the activation of an appropriate substrate. For example, appropriate cells (*i.e.*, cells that express DSP-3) may be transfected with a substrate-dependent promoter linked to a reporter gene. In such a system, expression of the reporter gene (which may be readily detected using methods well known to those of ordinary skill in the art) depends upon activation of substrate. Dephosphorylation of substrate may be detected based on a decrease in reporter activity. Candidate modulating agents may be added to such a system, as described above, to evaluate their effect on DSP-3 activity.

The present invention further provides methods for identifying a molecule that interacts with, or binds to, DSP-3. Such a molecule generally associates with DSP-3 with an affinity constant ( $K_a$ ) of at least  $10^4$ , preferably at least  $10^5$ , more preferably at least  $10^6$ , still more preferably at least  $10^7$  and most preferably at least  $10^8$ . Affinity constants may be determined using well known techniques. Methods for identifying interacting molecules may be used, for example, as initial screens for modulating agents, or to identify factors that are involved in the *in vivo* DSP-3 activity.

Techniques for substrate trapping, for example using DSP-3 variants or substrate trapping mutants as described above, are also contemplated according to certain embodiments provided herein. In addition to standard binding assays, there are many other techniques that are well known for identifying interacting molecules, including yeast two-hybrid screens, phage display and affinity techniques. Such techniques may be performed using routine protocols, which are well known to those having ordinary skill in the art (*see, e.g., Bartel et al., In Cellular Interactions in Development: A Practical Approach*, D.A. Harley, ed., Oxford University Press (Oxford, UK), pp. 153-179, 1993). Within these and other techniques, candidate interacting proteins (*e.g.,* putative DSP-3 substrates) may be phosphorylated prior to assaying for the presence of DSP-3-binding or interacting proteins.

Within other aspects, the present invention provides animal models in which an animal either does not express a functional DSP-3, or expresses an altered DSP-3. Such animals may be generated using standard homologous recombination strategies. Animal models generated in this manner may be used to study activities of DSP-3 polypeptides and modulating agents *in vivo*.

#### METHODS FOR DEPHOSPHORYLATING A SUBSTRATE

In another aspect of the present invention, a DSP-3 polypeptide may be used for dephosphorylating a substrate of DSP-3 as provided herein. In one embodiment, a substrate may be dephosphorylated *in vitro* by incubating a DSP-3 polypeptide with a substrate in a suitable buffer (*e.g.,* Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 1 mg/mL bovine serum albumin) for 10 minutes at 30°C. Any compound that can be dephosphorylated by DSP-3, such as a MAP-kinase, may be used as a substrate. In general, the amounts of the reaction components may range from about 50 pg to about 50 ng of DSP-3 polypeptide and from about 10 ng to about 10 µg of substrate. Dephosphorylated substrate may then be purified, for example, by affinity techniques and/or gel electrophoresis. The extent of substrate dephosphorylation may generally be monitored by adding [ $\gamma$ -<sup>32</sup>P]labeled substrate to a test aliquot, and evaluating the level of substrate dephosphorylation as described herein.

## METHODS FOR MODULATING CELLULAR RESPONSES

Modulating agents may be used to modulate, modify or otherwise alter (e.g., increase or decrease) cellular responses such as cell proliferation, differentiation and survival, in a variety of contexts, both *in vivo* and *in vitro*. In general, to so modulate (e.g., increase or decrease in a statistically significant manner) such a response, a cell is contacted with an agent that modulates DSP-3 activity, under conditions and for a time sufficient to permit modulation of DSP-3 activity. Agents that modulate a cellular response may function in any of a variety of ways. For example, an agent may modulate a pattern of gene expression (i.e., may enhance or inhibit expression of a family of genes or genes that are expressed in a coordinated fashion). A variety of hybridization and amplification techniques are available for evaluating patterns of gene expression. Alternatively, or in addition, an agent may effect apoptosis or necrosis of the cell, and/or may modulate the functioning of the cell cycle within the cell. (See, e.g., Ashkenazi et al., 1998 *Science*, 281:1305; Thornberry et al., 1998 *Science* 281:1312; Evan et al., 1998 *Science* 281:1317; Adams et al., 1998 *Science* 281:1322; and references cited therein.)

Cells treated as described above may exhibit standard characteristics of cells having altered proliferation, differentiation or survival properties. In addition, such cells may (but need not) display alterations in other detectable properties, such as contact inhibition of cell growth, anchorage independent growth or altered intercellular adhesion. Such properties may be readily detected using techniques with which those having ordinary skill in the art will be familiar.

## THERAPEUTIC METHODS

One or more DSP-3 polypeptides, modulating agents and/or polynucleotides encoding such polypeptides and/or modulating agents may also be used to modulate DSP-3 activity in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a condition associated with DSP-3 activity or may be free of detectable disease. Accordingly, the treatment may be

of an existing disease or may be prophylactic. Conditions associated with DSP-3 activity include any disorder associated with cell proliferation, including cancer, graft-versus-host disease (GVHD), autoimmune diseases, allergy or other conditions in which immunosuppression may be involved, metabolic diseases, abnormal cell growth or proliferation and cell cycle abnormalities. Certain such disorders involve loss of normal MAP-kinase phosphatase activity, leading to uncontrolled cell growth. DSP-3 polypeptides, and polynucleotides encoding such polypeptides, can be used to ameliorate such disorders. Activators of DSP-3 may also be used to treat certain disorders, including Duchenne Muscular Dystrophy.

For administration to a patient, one or more polypeptides, polynucleotides and/or modulating agents are generally formulated as a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Such compositions may be in the form of a solid, liquid or gas (aerosol). Alternatively, compositions of the present invention may be formulated as a lyophilizate or compounds may be encapsulated within liposomes using well known technology. Pharmaceutical compositions within the scope of the present invention may also contain other components, which may be biologically active or inactive. Such components include, but are not limited to, buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, stabilizers, dyes, flavoring agents, and suspending agents and/or preservatives.

Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Carriers for therapeutic use are well known, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro ed. 1985). In general, the type of carrier is selected based on the mode of administration. Pharmaceutical compositions may be formulated for any appropriate manner of administration,

including, for example, topical, oral, nasal, intrathecal, rectal, vaginal, sublingual or parenteral administration, including subcutaneous, intravenous, intramuscular, intrasternal, intracavernous, intrameatal or intraurethral injection or infusion. For parenteral administration, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose, ethyl cellulose, glucose, sucrose and/or magnesium carbonate, may be employed.

A pharmaceutical composition (*e.g.*, for oral administration or delivery by injection) may be in the form of a liquid (*e.g.*, an elixir, syrup, solution, emulsion or suspension). A liquid pharmaceutical composition may include, for example, one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. The use of physiological saline is preferred, and an injectable pharmaceutical composition is preferably sterile.

The compositions described herein may be formulated for sustained release (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain an agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the

formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

5 For pharmaceutical compositions comprising a polynucleotide encoding a DSP-3 polypeptide and/or modulating agent (such that the polypeptide and/or modulating agent is generated *in situ*), the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, and bacterial, viral and mammalian expression systems. Techniques for  
10 incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

15 Within a pharmaceutical composition, a DSP-3 polypeptide, polynucleotide or modulating agent may be linked to any of a variety of compounds. For example, such an agent may be linked to a targeting moiety (*e.g.*, a monoclonal or polyclonal antibody, a protein or a liposome) that facilitates the delivery of the agent to the target site. As used herein, a "targeting moiety" may be any substance (such as a  
20 compound or cell) that, when linked to an agent enhances the transport of the agent to a target cell or tissue, thereby increasing the local concentration of the agent. Targeting moieties include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent  
25 thereof. Examples of antibody fragments are F(ab')<sub>2</sub>, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Targeting moieties may be selected based on the cell(s) or tissue(s) toward which the agent is expected to exert  
30 a therapeutic benefit.



Pharmaceutical compositions may be administered in a manner appropriate to the disease to be treated (or prevented). An appropriate dosage and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease, the particular  
5 form of the active ingredient and the method of administration. In general, an appropriate dosage and treatment regimen provides the agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (*e.g.*, an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival). For prophylactic use, a dose should be sufficient to prevent, delay the  
10 onset of or diminish the severity of a disease associated with cell proliferation.

Optimal dosages may generally be determined using experimental models and/or clinical trials. In general, the amount of polypeptide present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 0.01  $\mu\text{g}$  to about 100  $\mu\text{g}$  per kg of host, typically from about 0.1  $\mu\text{g}$  to about 10  $\mu\text{g}$ . The use of the minimum  
15 dosage that is sufficient to provide effective therapy is usually preferred. Patients may generally be monitored for therapeutic or prophylactic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those having ordinary skill in the art. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg  
20 animal.

The following Example is offered by way of illustration and not by way of limitation.

## EXAMPLE

Cloning and Sequencing cDNA Encoding DSP-3

5 This Example illustrates the cloning of a cDNA molecule encoding human DSP-3.

A conserved sequence motif surrounding the active site domain of dual-specificity phosphatases was identified as follows: Dual specificity phosphatases belong to the larger family of protein tyrosine phosphatases (PTPs) that share a conserved catalytic domain containing a cysteine residue situated N-terminal to a stretch of five variable amino acids followed by an arginine residue (Fauman et al.,  
10 *Trends In Bioch. Sci.* 21:413-417, 1996). DSPs typically contain a PTP active site motif but lack sequence homology to PTPs in other regions (Jia, *Biochem. and Cell Biol.* 75:17-26, 1997). There is, however, no reported consensus sequence that is conserved among DSPs, nor is a consensus region apparent from examination of the  
15 known DSP sequences such as those referred to above. To derive a longer consensus DSP amino acid sequence motif that would be useful for the identification of new DSP family members, multiple known human dual-specificity phosphatases sequences were aligned and compared. An alignment of eight amino acid sequences derived from eight human DSPs having MAP-kinase phosphatase activity yielded a conserved homology  
20 region consisting of a 23-amino acid peptide sequence containing the PTP active site signature motif. Thus, a candidate peptide having the sequence:

GRVLVHCQAGISRSGTNILAYLM

SEQ ID NO:4

25 was used to search the Expressed Sequence Tag database (Nat. Center for Biol. Information, [www.ncbi.nlm.nih.gov/dbEST](http://www.ncbi.nlm.nih.gov/dbEST)). The search employed an algorithm (tblastn) capable of reverse translation of the candidate peptide with iterations allowing for genetic code degeneracy within default parameters. The search results identified the EST AA374753, as well as AA411671 and H82446, as candidate MAP-kinase  
30 phosphatase sequences. The ESTs did not include a complete coding region of an

expressed gene such as a gene encoding a DSP-3 having MAP-kinase phosphatase activity, nor were the sense strand and open reading frame identified.

To obtain a full length coding region, human skeletal muscle cDNA was screened in 5' and 3' RACE (rapid amplification of cDNA ends) reactions as described (Frohman et al., *Proc. Nat. Acad. Sci. USA* 85:8998, 1988; Ohara et al., *Proc. Nat. Acad. Sci. USA* 86:5673, 1989; Loh et al., *Science* 243:217, 1989) using 5'/3' RACE kits (Boehringer Mannheim, Indianapolis, IN; Clontech, Palo Alto, CA; Life Technologies, Gaithersburg, MD) according to the supplier's instructions. Sequence information immediately adjacent to the conserved sequence motif was used in the 5' and 3' RACE reactions with human skeletal muscle cDNA, using the following primers (SEQ ID NOs:5 to 8):

DSP3-SP1: 5'—GAC CTC ATG CTT CTC AAA CTC CTG—3' SEQ ID NO:5

DSP3-SP1.5: 5'—CGA TCA CCA GTG TCA CGC TCC—3' SEQ ID NO:6

15 DSP3-SP3: 5'—CAG AAT ATG TGT CAC CTT GTT CTT GC—3' SEQ ID NO:7

DSP3-SP5: 5'—GCA AGA ACA AGG TGA CAC ATA TTC TG—3' SEQ ID NO:8

Full length cDNA lacking only the initiation and stop codons was obtained using the following primers (SEQ ID NOS:9-10):

20

DSP3-ex5': 5'--GGG AAT GGG ATG AAC AAG ATC CTG CCC G--3'

SEQ ID NO:9

DSP3-ex3': 5'-CAGTCTTCTGAGAAAGGCCAGAACTTCAGAATTCCT-3'

25

SEQ ID NO:10

A cDNA (Figure 1; SEQ ID NO:1) encoding a protein of 184 amino acids (Figure 2; SEQ ID NO:2) was identified as DSP-3. This sequence has significant homology to other MAP-kinase phosphatases (Figure 3). The identified cDNA contains the 552 base pair coding region, as well as associated 5' and 3' untranslated

30

sequences. The active site domain for DSP-3 was localized to the region encoded by nucleotides beginning at position 258 of SEQ ID NO:1.

Semiquantitative RT-PCR analyses were performed. These analyses showed significantly higher levels of DSP-3 mRNA in skeletal muscle tissues.

## EXAMPLE 2

### DSP-3 EXPRESSION IN HUMAN TISSUES

In this example, a DSP-3 encoding nucleic acid sequence is shown to hybridize to human polyA<sup>+</sup> RNA from various tissue sources. Full length DSP-3 encoding cDNA (SEQ ID NO:1) was <sup>32</sup>P-labeled by the random primer method as described in Ausubel et al. (1998 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA) for use as a nucleic acid hybridization probe. The probe was hybridized to blots containing human polyA<sup>+</sup> RNA derived from multiple human tissues, normalized for the amount of detectable  $\beta$ -actin mRNA (Fig. 4, Cat. No. 7759-1; Clontech, Inc., Palo Alto, CA). Blots underwent prehybridization for 30 min at 68°C in Express Hyb<sup>TM</sup> solution (Clontech), and then were hybridized with the labeled probe for 1 hour at 68°C in Express Hyb<sup>TM</sup> solution. The blots were next washed for 40 min at room temperature in 2X SSC, 0.05% SDS, followed by a second wash for 40 min at 50°C in 0.1X SSC, 0.1% SDS. Blots were air-dried and then exposed to Hyperfilm MP<sup>TM</sup> autoradiographic film (Amersham Life Sciences, Arlington Hts, IL) overnight. Results are shown in Figure 4, in which the human tissue sources for the RNAs were as follows: Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas. Particularly pronounced DSP-3 expression was detected in human heart, liver, skeletal muscle and pancreas, with expression also detected in other tissues..

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.

Claims

1. An isolated polypeptide having the sequence of DSP-3 recited in SEQ ID NO:2, or a variant thereof that differs in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide retains the ability to dephosphorylate an activated MAP-kinase.
2. An isolated polynucleotide that encodes at least ten consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2.
3. An isolated polynucleotide that encodes at least fifteen consecutive amino acids of a polypeptide having a sequence corresponding to SEQ ID NO:2.
4. An expression vector comprising a polynucleotide according to claim 2 or 3.
5. A host cell transformed or transfected with an expression vector according to claim 4.
6. An isolated polynucleotide that encodes a polypeptide according to claim 1.
7. A polynucleotide according to claim 6, comprising the sequence recited in SEQ ID NO:1.
8. An expression vector comprising a polynucleotide according to claim 6.
9. A host cell transformed or transfected with an expression vector according to claim 8.

10. An antisense polynucleotide comprising at least 15 consecutive nucleotides complementary to a polynucleotide according to claim 6.

11. An isolated polynucleotide that detectably hybridizes to the complement of the sequence recited in SEQ ID NO:1 under conditions that include a wash in 0.1X SSC and 0.1% SDS at 50 °C for 15 minutes.

12. An expression vector comprising a polynucleotide according to claim 10 or claim 11.

13. A host cell transformed or transfected with an expression vector according to claim 12.

14. A method of producing a DSP-3 polypeptide, comprising the steps of:  
(a) culturing a host cell according to claim 9 under conditions that permit expression of the DSP-3 polypeptide; and  
(b) isolating DSP-3 polypeptide from the host cell culture.

15. An isolated antibody, or antigen binding fragment thereof, that specifically binds to a DSP-3 polypeptide having the sequence of SEQ ID NO:2.

16. An antibody or fragment thereof according to claim 15, wherein the antibody is a monoclonal antibody.

17. A pharmaceutical composition comprising an antibody or fragment thereof according to claim 15 in combination with a physiologically acceptable carrier.

18. A method for detecting DSP-3 expression in a sample, comprising:  
(a) contacting a sample with an antibody or an antigen-binding fragment thereof according to claim 15, under conditions and for a time sufficient to allow formation of an antibody/DSP-3 complex; and

(b) detecting the level of antibody/DSP-3 complex, and therefrom detecting the presence of DSP-3 in a sample.

19. A method according to claim 18, wherein the antibody is linked to a support material.

20. A method according to claim 18, wherein the antibody is linked to a detectable marker.

21. A method according to claim 18, wherein the sample is a biological sample obtained from a patient.

22. A method for detecting DSP-3 expression in a sample, comprising:

(a) contacting a sample with an antisense polynucleotide according to claim 10 or claim 11; and

(b) detecting in the sample an amount of DSP-3 polynucleotide that hybridizes to the antisense polynucleotide, and therefrom detecting DSP-3 expression in the sample.

23. A method according to claim 22, wherein the amount of DSP-3 polynucleotide that hybridizes to the antisense polynucleotide is determined using polymerase chain reaction.

24. A method according to claim 22, wherein the amount of DSP-3 polynucleotide that hybridizes to the antisense polynucleotide is determined using a hybridization assay.

25. A method according to claim 22, wherein the sample comprises an RNA or cDNA preparation.

26. A method for screening for an agent that modulates DSP-3 activity, comprising the steps of:

(a) contacting a candidate agent with a polypeptide according to claim 1, under conditions and for a time sufficient to permit interaction between the polypeptide and candidate agent; and

(b) subsequently evaluating the ability of the polypeptide to dephosphorylate a DSP-3 substrate, relative to a predetermined ability of the polypeptide to dephosphorylate the DSP-3 substrate in the absence of candidate agent;

and therefrom identifying an agent that modulates DSP-3 activity.

27. A method according to claim 26, wherein the DSP-3 substrate is a MAP-kinase.

28. A method according to claim 26, wherein the candidate agent is a small molecule.

29. A method according to claim 26, wherein the small molecule is present within a combinatorial library.

30. A method for screening for an agent that modulates DSP-3 activity, comprising the steps of:

(a) contacting a candidate agent with a cell comprising a DSP-3 promoter operably linked to a polynucleotide encoding a detectable transcript or protein, under conditions and for a time sufficient to permit interaction between the promoter and candidate agent; and

(b) subsequently evaluating the expression of the polynucleotide, relative to a predetermined level of expression in the absence of candidate agent;

and therefrom identifying an agent that modulates DSP-3 activity.

31. A method according to claim 30, wherein the polynucleotide encodes a DSP-3 polypeptide.



32. A method according to claim 30, wherein the polynucleotide encodes a reporter protein.

33. A method for modulating a proliferative response in a cell, comprising contacting a cell with an agent that modulates DSP-3 activity.

34. A method for modulating differentiation of a cell, comprising contacting a cell with an agent that modulates DSP-3 activity.

35. A method for modulating survival of a cell, comprising contacting a cell with an agent that modulates DSP-3 activity.

36. A method according to any one of claims 33-35, wherein the agent modulates a pattern of gene expression.

37. A method according to any one of claims 33-35, wherein the cell displays contact inhibition of cell growth.

38. A method according to any one of claims 33-35, wherein the cell displays anchorage independent growth.

39. A method according to any one of claims 33-35, wherein the cell displays an altered intercellular adhesion property.

40. A method according to claim 35, wherein the agent modulates apoptosis.

41. A method according to claim 35, wherein the agent modulates the cell cycle.

42. A method according to claim 32, wherein the cell is present within a patient.

43. A method for treating a patient afflicted with a disorder associated with DSP-3 activity, comprising administering to a patient a therapeutically effective amount of an agent that modulates DSP-3 activity.

44. A method according to claim 43, wherein the disorder is selected from the group consisting of Duchenne muscular dystrophy, cancer, graft-versus-host disease, autoimmune diseases, allergies, metabolic diseases, abnormal cell growth, abnormal cell proliferation and cell cycle abnormalities.

45. A DSP-3 substrate trapping mutant polypeptide that differs from the sequence recited in SEQ ID NO:2 in one or more amino acid deletions, additions, insertions or substitutions at no more than 50% of the residues in SEQ ID NO:2, such that the polypeptide binds to a substrate with an affinity that is not substantially diminished relative to DSP-3, and such that the ability of the polypeptide to dephosphorylate a substrate is reduced relative to DSP-3.

46. A substrate trapping mutant polypeptide according to claim 45, wherein the polypeptide contains a substitution at position 57 or position 88 of SEQ ID NO:2.

47. A method for screening a molecule for the ability to interact with DSP-3, comprising the steps of:

(a) contacting a candidate molecule with a polypeptide according to claim 1 under conditions and for a time sufficient to permit the candidate molecule and polypeptide to interact; and

(b) detecting the presence or absence of binding of the candidate molecule to the polypeptide, and therefrom determining whether the candidate molecule interacts with DSP-3.

48. A method according to claim 47, wherein the step of detecting comprises an affinity purification step.

49. A method according to claim 47, wherein the step of detecting comprises a yeast two hybrid screen or a screen of a phage display library.

**DSP-3, encoded by 552 base pairs**

```
1  CCCCCGCCGCT CCTCCTCCCT GTAACATGCC ATAGTGCGCC TGC GACCACA CGGCCGGGGC
61 GCTAGCGTTC GCCTTCAGCC ACCATGGGGA ATGGGATGAA CAAGATCCTG CCCGGCCTGT
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181 ATATTCTGTC TGTCCACGAT AGTGCCAGGC CTATGTTGGA GGGAGTTAAA TACCTGTGCA
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361 CCAGGAGCGT GACACTGGTG ATCGCATACA TCATGACCGT CACTGACTTT GGCTGGGAGG
421 ATGCCCTGCA CACCGTGCGT GCTGGGAGAT CCTGTGCCAA CCCCAACGTG GGCTTCCAGA
481 GACAGCTCCA GGAGTTTGAG AAGCATGAGG TCCATCAGTA TCGGCAGTGG CTGAAGGAAG
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721 TATCCAGTAG TGATTTGTAA ACTTGTTTTT CATTTGAAGC TGAATATATA CGTAGTCATG
781 TTTATGTTGA GAACTAAGGA TATTCTTTAG CAAGAGAAA TATTTTCCCC TTATCCCCAC
841 TGCTGTGGAG GTTCTGTAC CTCGCTTGGA TGCCTGTAAG GATCCCGGGA GCCTTGCCGC
901 ACTGCCTTGT GGGTGGCTTG GCGCTC
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**FIG. 1**

**Translated full length protein, 184 amino acids**

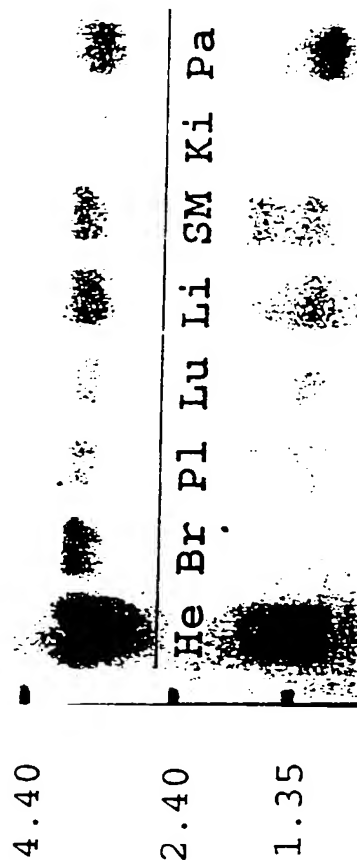
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GRSCANPNVGFQRQLQEFKHEVHQYRQWLKEEYGESPLQDAEEAKNILAAPGILKFWAF  
LRRL\*

**FIG. 2**



# DSP-3

## • Northern blot analysis



- He = Heart
- Br = Brain
- Pl = Placenta
- Lu = Lung
- Li = Liver
- SM = Skeletal Muscle
- Ki = Kidney
- Pa = Pancreas

FIGURE 4

## SEQUENCE LISTING

<110> Ceptyr, Inc.  
Luche, Ralf M.  
Wei, Bo

<120> DSP-3 DUAL-SPECIFICITY PHOSPHATASE

<130> 200125.40801PC

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His Ile Leu Ser Val His Asp Ser Ala Arg Pro Met Leu Glu Gly Val
      35             40            45

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Lys Lys Arg Val Arg Leu Glu Glu Ala Phe Glu Phe Val Lys Gln Arg
      130          135          140
Arg Ser Ile Ile Ser Pro Asn Phe Ser Phe Met Gly Gln Leu Leu Gln
145          150          155          160
Phe Glu Ser Gln Val Leu Ala Thr Ser
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Ser Tyr Arg Pro Ala Tyr Asp Gln Gly Gly Pro Val Glu Ile Leu Pro
      20          25          30
Phe Leu Tyr Leu Gly Ser Ala Tyr His Ala Ser Lys Cys Glu Phe Leu
      35          40          45
Ala Asn Leu His Ile Thr Ala Leu Leu Asn Val Ser Arg Arg Thr Ser
      50          55          60
Glu Ala Cys Met Thr His Leu His Tyr Lys Trp Ile Pro Val Glu Asp
65          70          75          80
Ser His Thr Ala Asp Ile Ser Ser His Phe Gln Glu Ala Ile Asp Phe
      85          90          95
Ile Asp Cys Val Arg Glu Lys Gly Gly Lys Val Leu Val His Cys Glu
      100          105          110
Ala Gly Ile Ser Arg Ser Pro Thr Ile Cys Met Ala Tyr Leu Met Lys
      115          120          125
Thr Lys Gln Phe Arg Leu Lys Glu Ala Phe Asp Tyr Ile Lys Gln Arg
      130          135          140
Arg Ser Met Val Ser Pro Asn Phe Gly Phe Met Gly Gln Leu Leu Gln
145          150          155          160
Tyr Glu Ser Glu Ile Leu Pro Ser Thr Pro Asn
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<210> 18  
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Thr Pro Arg Ile Tyr Val Gly Asn Ala Ser Val Ala Gln Asp Ile Pro
      35          40          45
Lys Leu Gln Lys Leu Gly Ile Thr His Val Leu Asn Ala Ala Glu Gly
      50          55          60
Arg Ser Phe Met His Val Asn Thr Asn Ala Asn Phe Tyr Lys Asp Ser
65          70          75          80

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Gly Ile Thr Tyr Leu Gly Ile Lys Ala Asn Asp Thr Gln Glu Phe Asn  
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 Leu Ser Ala Tyr Phe Glu Arg Ala Ala Asp Phe Ile Asp Gln Ala Leu  
                   100                  105                  110  
 Ala Gln Lys Asn Gly Arg Val Leu Val His Cys Arg Glu Gly Tyr Ser  
                   115                  120                  125  
 Arg Ser Pro Thr Leu Val Ile Ala Tyr Leu Met Met Arg Gln Lys Met  
                   130                  135                  140  
 Asp Val Lys Ser Ala Leu Ser Ile Val Arg Gln Asn Arg Glu Ile Gly  
 145                  150                  155                  160  
 Pro Asn Asp Gly Phe Leu Ala Gln Leu Cys Gln Leu Asn Asp Arg Leu  
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 Ala Lys Glu Gly  
                   180

<210> 19

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<212> PRT

<213> Homo sapien

<400> 19

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 Phe Lys Asp Ala Arg Asp Ala Glu Gln Leu Ser Lys Asn Lys Val Thr  
                   20                  25                  30  
 His Ile Leu Ser Val His Asp Ser Ala Arg Pro Met Leu Glu Gly Val  
                   35                  40                  45  
 Lys Tyr Leu Cys Ile Pro Ala Ala Asp Ser Pro Ser Thr Arg His Phe  
   50                  55                  60  
 Lys Glu Ser Ile Lys Phe Ile His Glu Cys Arg Leu Arg Gly Glu Ser  
 65                  70                  75                  80  
 Cys Leu Val His Cys Leu Ala Gly Val Ser Arg Ser Val Thr Leu Val  
                   85                  90                  95  
 Ile Ala Tyr Ile Met Thr Val Thr Asp Phe Gly Trp Glu Asp Ala Leu  
                   100                  105                  110  
 His Thr Val Arg Ala Gly Arg Ser Cys Ala Asn Pro Asn Val Gly Phe  
                   115                  120                  125  
 Gln Arg Gln Leu Gln Glu Phe Glu Lys His Glu Val His Gln Tyr Arg  
                   130                  135                  140  
 Gln  
 145

## INTERNATIONAL SEARCH REPORT

Int. l. Application No.

/US 00/10868

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/52 C12N9/16 G01N33/53 G01N33/68 C12Q1/68  
 C12N15/11 C07K16/40 A61K39/395 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N G01N C12Q C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>WO 00 06728 A (INCYTE PHARMA INC (US)            HILLMAN LAL TANG CORLEY GUEGLER BAUGHN ET            AL) 10 February 2000 (2000-02-10)            page 15, line 3,4            page 26, paragraph 1            SEQ ID NO: 27, 58            page 51; table 1            page 60; table 2            page 65; table 3            page 72; table 4            page 75 -page 76; claims</p> <p style="text-align: center;">--- -/-</p>	<p>1-29, 33,            43, 44,            47-49</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

18 September 2000

Date of mailing of the international search report

29/09/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Macchia, G



## INTERNATIONAL SEARCH REPORT

Int lional Application No

P S 00/10868

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL NUCLEOTIDE SEQUENCE 'Online! Homo sapiens ID: HS1206913; AC: AA411671, 4 May 1997 (1997-05-04) "99.6% identity with SEQ ID NO:1 from nt.143 to nt.422. Similarity with a Dual Specificity Phosphatase" XP002147544 cited in the application abstract</p> <p>---</p>	2,3,11
X	<p>DATABASE EMBL NUCLEOTIDE SEQUENCE 'Online! Homo sapiens ID: HS1207233; AC: AA410486, 5 May 1997 (1997-05-05) "99.6% identity with SEQ ID NO:1 from nt.405 to nt.851" XP002147545 abstract</p> <p>---</p>	2,3,11
X	<p>DATABASE EMBL NUCLEOTIDE SEQUENCE 'Online! Mus musculus ID: MAA3595; AC: AA103595, 3 October 1996 (1996-10-03) "88.8% identity with SEQ ID NO:1 from nt.78 to nt.381" XP002147546 abstract</p> <p>---</p>	2,3,11
A	<p>WO 99 02704 A (COLD SPRING HARBOR LAB (US) TONKS NICHOLAS K (US); MYERS MICHAEL P (US) 21 January 1999 (1999-01-21) abstract</p> <p>---</p>	
A	<p>KEYSE S.M.: "An emerging family of Dual Specificity MAP Kinase Phosphatases" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1265, 1995, pages 152-160, XP000196716 ISSN: 0167-4889 abstract</p> <p>-----</p>	

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

/US 00/10868

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0006728	A	10-02-2000	AU 5134999 A	21-02-2000
WO 9902704	A	21-01-1999	AU 8479498 A	08-02-1999

**DSP-3, encoded by 552 base pairs**

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1  CCCCCGGGGT GCTCCTCCCT GTAACATGCC ATAGTGGGCG TGGGACCACA CCGCCGGGGC
61  GGTAGCGTTC GCCTTCAGCC ACCATGGGGA ATGGGATGAA CAGGATCCTG CCGGGCCTGT
121 ACATGGGCAA CTTCAAAGAT GGCAGAGAGC CGGAACGATT GAGCAGAAAC AAGGTGACAC
181 ATATTCTGTC TGTCCACGAT AGTGCCAGGC CTATGTTGGA GGGAGTTAAA TACCTGTGCA
241 TCCGAGCAGC GGATTCACCA TCYCAAACC TGACAAGACA TTCAAAGAA AGTATTAAT
301 TCATTCAAGA GTGCGCGCTC CGCGGTGAGA CCTGCCCTGT ACACTGCTG GCCGGGGTCT
361 CCAGGAGCCT GACACTGGTG ATCGCATACA TCATGACCGT CACTGACTTT GGCTGGGAGG
421 ATGCCCCGCA CACCGTGCGT GCTGGGAGAT CCTGTGCCAA CCCCAGGTG GGCTTCCAGA
481 GACAGCTCCA GGAGTTTGAG AAGCATGAGG TCCATCAGTA TCGGCAGTGG CTGAAGGAAG
541 AATATGGAGA GAGCCCTTTG CAGGATGCAG AAGAAGCCAA AAACATTCTG GCGCTCCAG
601 GAATTCTGAA GTTCTGGGEC TTTCTCAGAA GACTGTAAAG TACCTGAAT TTCTGAATA
661 TTGCAGACCC GCAGATTTA GGCTGCTGCT GCCAAAAGA AAASCAACAT AGAGTTTAAG
721 TATCCAGTAG TGATTTGTAA ACTTGTTTTT CATTTGAAGC TGAATATATA CGTAGTCATG
781 TTTATGTTGA GAACTAAGGA TATTCTTTTG CAGGAGAAAA TATTTTCCC TTATCCCCAC
841 TGCTGTGGAG GTTTCTGTAC CTCGCTTGGG TGCTGTAAAG GATCCCCGGA GCCTTGCCGC
901 ACTGCCCTGT GGGTGGCTTG GCGCTC

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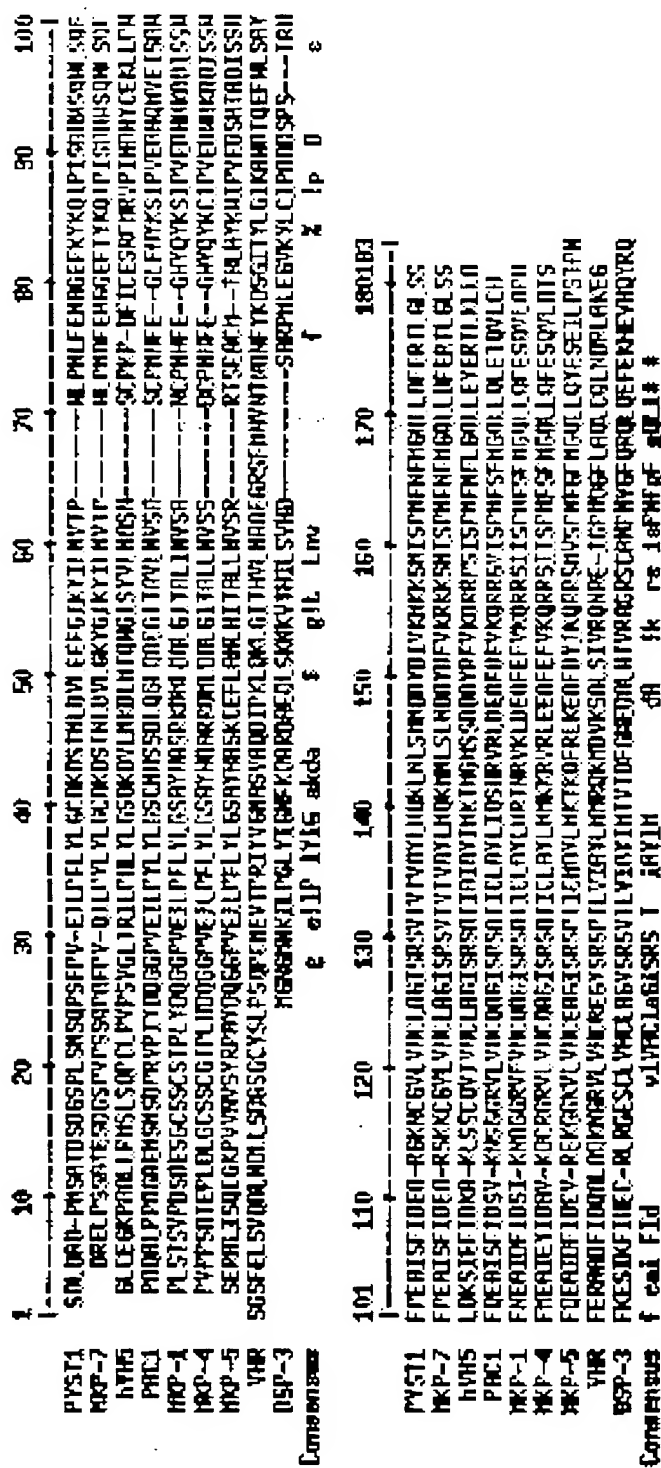
**FIG. 1**

**Translated full length protein, 154 amino acids**

MGNGMKNKILPGLYIGNFKDARDAEQLSKNEVTRILSVHDSARPMLEGVKYLCIPAADSPS  
QNLTRHFKESEIKFIHECRLRGESCLVHCLAGVSRSVTLVIAYIMTVTDFGWEDALHTVRA  
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LRSL\*

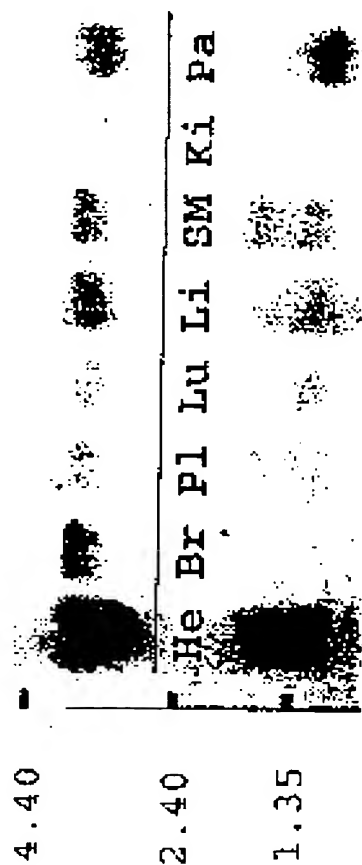
**FIG. 2**

FIG. 3



# DSP-3

## • Northern blot analysis



- He = Heart
- Br = Brain
- Pl = Placenta
- Lu = Lung
- Li = Liver
- SM = Skeletal Muscle
- Ki = Kidney
- Pa = Pancreas

FIGURE 4

## SEQUENCE LISTING

<110> Ceptyr, Inc.  
 Lucbe, Ralf M.  
 Wei, Bo

<120> DSP-3 DUAL-SPECIFICITY PHOSPHATASE

<130> 200125.40801PC

<140> PCT

<141> 2000-04-20

<160> 19

<170> FastSEQ for Windows Version 4.0

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<213> Homo sapien

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atctctgttc tgtccagat agtgcaggc ctatgttggg gggagtttaa tactgtgca    240
tccagcagc ggattcacca ttccaacc ccacaagaca ttccaagaa agtattaat    300
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ccaggagcgt gacactggtg atgcacata tcatgacct cactgacttt ggcaggaggg    420
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tttatgttga gaactaagga tattctttag caagagaaas tattttccc ttatccccc    840
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<212> PRT

<213> Homo sapien

<400> 2

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                20                25                30
His Ile Leu Ser Val His Asp Ser Ala Arg Pro Met Leu Glu Gly Val
          35                  40                  45

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Lys Tyr Leu Cys Ile Pro Ala Ala Asp Ser Pro Ser Gln Asn Leu Thr
 50                      55                      60
Arg His Phe Lys Glu Ser Ile Lys Phe Ile His Glu Cys Arg Leu Arg
 65                      70                      75                      80
Gly Glu Ser Cys Leu Val His Cys Leu Ala Gly Val Ser Arg Ser Val
 85                      90                      95
Thr Leu Val Ile Ala Tyr Ile Met Thr Val Thr Asp Phe Gly Trp Glu
100                      105                      110
Asp Ala Leu His Thr Val Arg Ala Gly Arg Ser Cys Ala Asn Pro Asn
115                      120                      125
Val Gly Phe Gln Arg Gln Leu Gln Glu Phe Glu Lys His Glu Val His
130                      135                      140
Gln Tyr Arg Gln Trp Leu Lys Glu Glu Tyr Gly Glu Ser Pro Leu Gln
145                      150                      155                      160
Asp Ala Glu Glu Ala Lys Asn Ile Leu Ala Ala Pro Gly Ile Leu Lys
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<212> PRT
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      DSP-3

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DSP-3

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 Leu Tyr Leu Gly Cys Ala Lys Asp Ser Thr Asn Leu Asp Val Leu Glu  
 35 40 45  
 Glu Phe Gly Ile Lys Tyr Ile Leu Asn Val Thr Pro Asn Leu Pro Asn  
 50 55 60  
 Leu Phe Glu Asn Ala Gly Glu Phe Lys Tyr Lys Gln Ile Pro Ile Ser  
 65 70 75 80  
 Asp His Trp Ser Gln Asn Leu Ser Gln Phe Phe Pro Glu Ala Ile Ser  
 85 90 95  
 Phe Ile Asp Glu Ala Arg Gly Lys Asn Cys Gly Val Leu Val His Cys  
 100 105 110  
 Leu Ala Gly Ile Ser Arg Ser Val Thr Val Thr Val Ala Tyr Leu Met  
 115 120 125  
 Gln Lys Leu Asn Leu Ser Met Asn Asp Ala Tyr Asp Ile Val Lys Met  
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 Lys Lys Ser Asn Ile Ser Pro Asn Phe Asn Phe Met Gly Gln Leu Leu  
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 <211> 168  
 <212> PRT  
 <213> Homo sapien

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 35 40 45  
 Gly Ile Lys Tyr Ile Leu Asn Val Thr Pro Asn Leu Pro Asn Ala Phe  
 50 55 60  
 Glu His Gly Gly Glu Phe Thr Tyr Lys Gln Ile Pro Ile Ser Asp His  
 65 70 75 80  
 Trp Ser Gln Asn Leu Ser Gln Phe Phe Pro Glu Ala Ile Ser Phe Ile  
 85 90 95  
 Asp Glu Ala Arg Ser Lys Lys Cys Gly Val Leu Val His Cys Leu Ala  
 100 105 110  
 Gly Ile Ser Arg Ser Val Thr Val Thr Val Ala Tyr Leu Met Gln Lys  
 115 120 125  
 Met Asn Leu Ser Leu Asn Asp Ala Tyr Asp Phe Val Lys Arg Lys Lys  
 130 135 140  
 Ser Asn Ile Ser Pro Asn Phe Asn Phe Met Gly Gln Leu Leu Asp Phe



Ser His Arg Val Arg Leu Asp Glu Ala Phe Asp Phe Val Lys Gln Arg  
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 145 150 155 160  
 Leu Glu Thr Gln Val Leu Cys His  
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<210> 15  
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 35 40 45  
 Asp Ala Leu Gly Ile Thr Ala Leu Ile Asn Val Ser Ala Asn Cys Pro  
 50 55 60  
 Asn His Phe Glu Gly His Tyr Gln Tyr Lys Ser Ile Pro Val Glu Asp  
 65 70 75 80  
 Asn His Lys Ala Asp Ile Ser Ser Trp Phe Asn Glu Ala Ile Asp Phe  
 85 90 95  
 Ile Asp Ser Ile Lys Asn Ala Gly Gly Arg Val Phe Val His Cys Gln  
 100 105 110  
 Ala Gly Ile Ser Arg Ser Ala Thr Ile Cys Leu Ala Tyr Leu Met Arg  
 115 120 125  
 Thr Asn Arg Val Lys Leu Asp Glu Ala Phe Glu Phe Val Lys Gln Arg  
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 Arg Ser Ile Ile Ser Pro Asn Phe Ser Phe Met Gly Gln Leu Leu Gln  
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 Phe Glu Ser Gln Val Leu Ala Pro His  
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<210> 16  
 <211> 169  
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 20 25 30  
 Phe Leu Tyr Leu Gly Ser Ala Tyr His Ala Ala Arg Arg Asp Met Leu  
 35 40 45  
 Asp Ala Leu Gly Ile Thr Ala Leu Leu Asn Val Ser Ser Asp Cys Pro  
 50 55 60  
 Asn His Phe Glu Gly His Tyr Gln Tyr Lys Cys Ile Pro Val Glu Asp  
 65 70 75 80  
 Asn His Lys Ala Asp Ile Ser Ser Trp Phe Met Glu Ala Ile Glu Tyr  
 85 90 95  
 Ile Asp Ala Val Lys Asp Cys Arg Gly Arg Val Leu Val His Cys Gln

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	115		120		125										
Lys	Lys	Arg	Val	Arg	Leu	Glu	Glu	Ala	Phe	Glu	Phe	Val	Lys	Gln	Arg
	130		135		140										
Arg	Ser	Ile	Ile	Ser	Pro	Asn	Phe	Ser	Phe	Met	Gly	Gln	Leu	Leu	Gln
	145		150		155		160								
Phe	Glu	Ser	Gln	Val	Leu	Ala	Thr	Ser							
			165												

&lt;210&gt; 17

&lt;211&gt; 171

&lt;212&gt; PRT

&lt;213&gt; Homo sapien

&lt;400&gt; 17

Ser	Glu	Arg	Ala	Leu	Ile	Ser	Gln	Cys	Gly	Lys	Pro	Val	Val	Asn	Val
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Ser	Tyr	Arg	Pro	Ala	Tyr	Asp	Gln	Gly	Gly	Pro	Val	Glu	Ile	Leu	Pro
			20				25					30			
Phe	Leu	Tyr	Leu	Gly	Ser	Ala	Tyr	His	Ala	Ser	Lys	Cys	Glu	Phe	Leu
	35					40					45				
Ala	Asn	Leu	His	Ile	Thr	Ala	Leu	Leu	Asn	Val	Ser	Arg	Arg	Thr	Ser
	50				55				60						
Glu	Ala	Cys	Met	Thr	His	Leu	His	Tyr	Lys	Trp	Ile	Pro	Val	Glu	Asp
65				70				75				80			
Ser	His	Thr	Ala	Asp	Ile	Ser	Ser	His	Phe	Gln	Glu	Ala	Ile	Asp	Phe
			85					90				95			
Ile	Asp	Cys	Val	Arg	Glu	Lys	Gly	Gly	Lys	Val	Leu	Val	His	Cys	Glu
	100						105					110			
Ala	Gly	Ile	Ser	Arg	Ser	Pro	Thr	Ile	Cys	Met	Ala	Tyr	Leu	Met	Lys
	115					120					125				
Thr	Lys	Gln	Phe	Arg	Leu	Lys	Glu	Ala	Phe	Asp	Tyr	Ile	Lys	Gln	Arg
	130				135					140					
Arg	Ser	Met	Val	Ser	Pro	Asn	Phe	Gly	Phe	Met	Gly	Gln	Leu	Leu	Gln
145				150				155						160	
Tyr	Glu	Ser	Glu	Ile	Leu	Pro	Ser	Thr	Pro	Asn					
			165					170							

&lt;210&gt; 18

&lt;211&gt; 180

&lt;212&gt; PRT

&lt;213&gt; Homo sapien

&lt;400&gt; 18

Ser	Gly	Ser	Phe	Glu	Leu	Ser	Val	Gln	Asp	Leu	Asn	Asp	Leu	Leu	Ser
1			5					10					15		
Asp	Gly	Ser	Gly	Cys	Tyr	Ser	Leu	Pro	Ser	Gln	Pro	Cys	Asn	Glu	Val
	20						25					30			
Thr	Pro	Arg	Ile	Tyr	Val	Gly	Asn	Ala	Ser	Val	Ala	Gln	Asp	Ile	Pro
	35					40					45				
Lys	Leu	Gln	Lys	Leu	Gly	Ile	Thr	His	Val	Leu	Asn	Ala	Ala	Glu	Gly
	50				55					60					
Arg	Ser	Phe	Met	His	Val	Asn	Thr	Asn	Ala	Asn	Phe	Tyr	Lys	Asp	Ser
65				70				75					80		

Gly Ile Thr Tyr Leu Gly Ile Lys Ala Asn Asp Thr Gln Glu Phe Asn  
                   85                  90                  95  
 Leu Ser Ala Tyr Phe Glu Arg Ala Ala Asp Phe Ile Asp Gln Ala Leu  
                   100                  105                  110  
 Ala Gln Lys Asn Gly Arg Val Leu Val His Cys Arg Glu Gly Tyr Ser  
                   115                  120                  125  
 Arg Ser Pro Thr Leu Val Ile Ala Tyr Leu Met Met Arg Gln Lys Met  
                   130                  135                  140  
 Asp Val Lys Ser Ala Leu Ser Ile Val Arg Gln Asn Arg Glu Ile Gly  
 145                  150                  155                  160  
 Pro Asn Asp Gly Phe Leu Ala Gln Leu Cys Gln Leu Asn Asp Arg Leu  
                   165                  170                  175  
 Ala Lys Glu Gly  
                   180

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<400> 19  
 Met Gly Asn Gly Met Asn Lys Ile Leu Pro Gly Leu Tyr Ile Gly Asn  
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 Phe Lys Asp Ala Arg Asp Ala Glu Gln Leu Ser Lys Asn Lys Val Thr  
                   20                  25                  30  
 His Ile Leu Ser Val His Asp Ser Ala Arg Pro Met Leu Glu Gly Val  
                   35                  40                  45  
 Lys Tyr Leu Cys Ile Pro Ala Ala Asp Ser Pro Ser Thr Arg His Phe  
                   50                  55                  60  
 Lys Glu Ser Ile Lys Phe Ile His Glu Cys Arg Leu Arg Gly Glu Ser  
 65                  70                  75                  80  
 Cys Leu Val His Cys Leu Ala Gly Val Ser Arg Ser Val Thr Leu Val  
                   85                  90                  95  
 Ile Ala Tyr Ile Met Thr Val Thr Asp Phe Gly Trp Glu Asp Ala Leu  
                   100                  105                  110  
 His Thr Val Arg Ala Gly Arg Ser Cys Ala Asn Pro Asn Val Gly Phe  
                   115                  120                  125  
 Gln Arg Gln Leu Gln Glu Phe Glu Lys His Glu Val His Gln Tyr Arg  
                   130                  135                  140  
 Gln  
 145